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HEMATOLOGIC VALUES FOR NORMAL HEALTHY MEN 16 TO 25 YEARS OF AGE*

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NUMEROUS investigations have been made in this country and in other countries during the past few years in an effort to determine the reliability of long-accepted blood values and to establish standard normal blood values for particular geographical areas. The greater number of studies have been concerned with the normal or mean values for the quantity of hemoglobin and the number of red blood cells for healthy persons. Only a relatively small number of studies have been made on the normal values for other blood elements. The present report is concerned with the results of complete hematologic examinations of healthy men. Reports in the literature of similar complete hematologic studies for men have not come to our attention.

Reviews, including normal values for all blood elements for adult men, are lacking, though reviews of the studies of normal values of some individual blood elements are found in the literature. Myers and Eddy,[1] and Nelson and Stoker,[2] have reviewed the literature on normal values for hemoglobin of men, while the latter, Andresen and Mugrage,[3] and Mugrage and Andresen[4] have reviewed the literature on normal numbers of red blood cells. The last-named authors have also presented a consideration of the values for the various corpuscular constants. Garrey and Bryan,[5] Osgood,[6] and Osgood and co-workers[7, 8] have reviewed the literature on leucocyte values, while Olef[9] has reviewed

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TABLE I

MEAN VALUES FOR HEMOGLOBIN, RED BLOOD CELLS, CELL VOLUME, AND CORPUSCULAR CONSTANTS REPORTED FOR MEN

OBSERVER	YEAR	PLACE	NUMBER	AGE	METHOD OF HB. DETERMINATIONS	HB. (GM. PER 100 C.C. BLOOD)	ERYTHROCYTES (MILLIONS PER C.MM. BLOOD)	PACKED R.B.C. VOLUME (PER CENT)	MEAN CORPUSCULAR VOLUME (C. MICRONS)	MEAN CORPUSCULAR HB. (MICROMICROGRAMS)	MEAN CORPUSCULAR HB. CONCENTRATION (PER CENT)
United States of America											
Haden[19]	1922	Kansas City	20	18-30	Van Slyke	15.83	5.08				
			20	30-50	Van Slyke	15.23	4.86				
Sackett[20]	1925	Kansas City	15		Wong	16.71	5.09				
Osgood[21]	1926	Portland	137	19-30	Osgood-Haskins	15.76	5.39	44.64			
Wintrobe and Miller[22]	1929	New Orleans	100	20-30	Newcomer	15.87	5.85	46.50			
Murphy, Lynch, and Howard[23]	1930	Boston	18		Osgood-Haskins	15.06	5.35	44.50			
Foster and Johnson[24]	1931	New Orleans	115	18-30	Van Slyke-Neill	15.63	5.26	44.40	85.92	30.02	34.77
Osgood, Haskins, and Trotman[25]	1932	Portland	196		Osgood-Haskins	15.80	5.40	44.79			
Wintrobe[11]	1932	New Orleans							87.00	29.50	35.00
Haden[26]	1933	Cleveland	70			15.34	4.95				
Sachs, Levine, and Appelsis[27]	1933	Omaha	100	20-25	Wong	14.93	5.00				
Goldhamer and Fritzell[14]	1933	Ann Arbor	100	12-17	Sahli	11.55	4.71				
Helmer and Emerson[28]	1934	Indianapolis	10	20-40	Van Slyke-Neill	15.29	5.58				
Broun and Briggs[29]	1934	St. Louis	23		Van Slyke-Neill protein iron and acid hematin	16.60	5.29	46.57			
Walters[16]	1934	Lawrence	100	20-30	Newcomer	15.12	4.84	46.50	96.50	31.40	32.40
Walters[17]	1934	Lawrence	10	18-25	Newcomer	16.08	5.26	49.90	95.00	30.6	32.10
Osgood[6]	1935	Portland	259	14-30	Osgood-Haskins	15.84	5.42	44.79*			
Sachs, Levine, and Fabian[30]	1935	Omaha	50	20-25	Wong	15.01	4.68				
Peterson and Peterson[31]	1935	Butte	75		Dare	86.78	5.17				
Andresen and Mugrage[3]	1936	Denver	40	20-45	Van Slyke-Neill	16.54	5.42	48.35	89.20	30.50	34.20
Nelson and Stoker[2]	1937	Lawrence	121	18-25	Van Slyke	14.71	5.09				
			350	18-65		15.03	5.11				
Mugrage and Andresen[4]	1938	Denver	20	17-19	Van Slyke-Neill	16.57	5.35	49.55	92.60	30.90	33.40
			20	19-21	Van Slyke-Neill	16.45	5.35	48.70	91.00	30.70	33.80
Myers and Eddy[1]	1939	Cleveland	111		Bing and Baker	15.66					

Europe											
Gram and Norgaard[32]	1923	Copenhagen	10	20-42	Autenrieth-Konigsberger	15.01	5.45	46.34			
Heilmeyer and Hausold[33]	1936	Jena	80		Barcroft photometer	15.90	5.06				
Bierring and Sorensen[34]	1936	Copenhagen	60		Van Slyke	14.90	5.07	44.90			
Dor[35]	1938	Liége		18-40	Van Slyke and spectrophotometer	14.72					
South America											
Orias[36]	1930	Buenos Aires†	321		Newcomer	14.28					
		Buenos Aires‡	82		Newcomer	15.30					
		Jujuy†	261		Newcomer	14.55					
		Mendoza†	260		Newcomer	14.56					
		Corrientes†	262		Newcomer	14.49					
Tenconi[37]	1931	Buenos Aires	50		Newcomer	14.80	5.30	43.17			
Australia											
Wardlaw, Barry, McDonald, and McIntyre[38]	1935	Sidney	26		Newcomer	15.83					
		Central Australia	13		Newcomer	14.95					
		Aborigines	25		Newcomer	15.11					
Philippines											
Chamberlain[39]	1911	Manila (soldiers)	702		Dare and von Fleishel	89.60%	5.20				
Navarro[40]	1937	Manila	104	19-39	Wong	14.11	5.16	43.00	84.00	27.00	32.00
India											
Sokhey, Gokhale, Malandkar, and Billimoria[41]	1937	Bombay	121	19-30	Van Slyke-Neill	15.37	5.11	41.72			
Shourie and Singh[42]	1938	Wellington	10	20-40	Bing and Baker	20.92					
Sankaran and Rajagopal[43]	1938	Madras	125	18-25	Bing and Baker	16.57					
Cropper and Kahali[44]	1938		Burmese	18-45	Hellige	14.38					
			Karens			14.69					
Napier and Majumdar[45]	1938	Calcutta	25	20-40	Hellige	12.60	5.05	42.18	84.93	25.14	29.72
Africa											
Liknaitzky[46]	1934	Johannesburg	60		Haldane-Gower	14.54	5.99				
Present study	1939	Honolulu	137	16-25	Acid-hematin photo-electric colorimeter	15.10	5.08	44.20	86.50	29.20	34.70

*Cell volume for 153 men 20 to 30 years of age.
†Conscripts.
‡Students.

studies of normal values for platelets. The results of studies of normal values for the various blood elements for men in the literature available to us are summarized in Tables I and II for the purpose of comparison with the results of investigations of mean blood values presented here.

SUBJECTS EXAMINED

The 137 men examined in this study were all university students, ranging in age from 16 to 25 years. All had resided in the Hawaiian Islands for at least two years, and the greater number of them were born there and had lived continuously in the Islands. The individuals were all of average good health, and none had had recent illness. They were of different racial groups, the number of persons in each age group, and as well the number in each racial group, are indicated in Tables III and IV. Honolulu is situated on the seashore, and the homes of its residents range from sea level to an altitude of approximately 1,000 feet.

METHODS OF STUDY

All blood samples of the subjects examined were of capillary blood, obtained from a deep puncture of the finger made with a sharp spring lancet, only the freely flowing blood being used. The hemglobin estimations for 31 men were made by the acid hematin method, employing a Bausch & Lomb colorimeter equipped with a standard hemoglobin color disk. The color disk was checked for accuracy during the course of the study. The remaining hemoglobin estimations were made with a Klett-Summerson photoelectric colorimeter employing the acid hematin method, the colorimeter being checked and standardized against the Van Slyke oxygen capacity method of hemoglobin determination.

Enumerations of erythrocytes, leucocytes, and platelets were made by the use of Thoma pipettes and Levy counting chambers, certified for accuracy by the United States Bureau of Standards. Hayem's solution and Tuerk's solution were used in diluting the blood samples for red and white blood cell counts made in the usual manner. The solution of Rees and Ecker was used for dilution of blood samples for platelets, the counts being made by the direct method. The volume of packed red blood cells was determined by the use of capillary hematocrit pipettes according to the method described by one of us (C. J. H.),[10] and calculations of the corpuscular constants were made by the use of the formulas suggested by Wintrobe.[11] Differential leucocyte counts were made from blood smears stained with Wright's stain and counterstained with Giemsa. Counts were made from three smears for each person, the average for the three counts being taken as the differential count for the person.

The data obtained in this study were submitted to statistical analysis, and mean values were determined for each blood element for each age group and racial group. Statistical constants were calculated in all instances. The results and conclusions presented in this paper are based on the statistical studies of these data.

OBSERVATIONS AND RESULTS

It has frequently been suggested that values for the different blood elements may vary for different racial groups. It has also been suggested that

TABLE II

MEAN VALUES FOR PLATELETS, LEUCOCYTES, AND DIFFERENTIAL LEUCOCYTE COUNTS REPORTED FOR MEN AND FOR MEN AND WOMEN

	YEAR	PLACE	NUMBER OF SUBJECTS	AGE	SEX	PLATELETS (THOUSANDS PER C.MM. BLOOD)	LEUCOCYTES (PER C.MM. BLOOD)	DIFFERENTIAL LEUCOCYTE COUNTS (PER CENT)					
								NEUTROPHILES	"STAB" CELLS	EOSINOPHILES	BASOPHILES	LYMPHOCYTES	MONOCYTES
Feinblatt[47]	1923	New York	80				7,379						
Stetson[48]	1927	Boston					2,700-14,000	51.04		2.31	0.85	36.04	9.62
							6,309						
Medlar[49]	1929						6,000- 9,000	50-64		1-30	0.5-1	25-39	6-9
Stammers[50]	1933	Johannesburg	171					54.20		1.88	0.69	39.72	4.24
Sachs, Levine, and Appelsis[27]	1933	Omaha	100	20-25			7,559						
Schweizer[51]	1933						4,700-11,500						
Jones and co-workers[52]	1933						6,280						
Peterson and Peterson[31]	1935	Butte	75		M		8,350						
			100		M & F		8,260	54.36		2.00	0.60	36.26	6.33
Bryan, Chastain, and Garrey[53]	1935	Nashville	18		M		5,618						
Osgood[6]	1935	Portland	269	19-30	M & F		7,447						
			378	14-30	M & F				0.78	1.90			4.18
			236	20-30	M & F			54.26				37.76	
			597	4-30	M & F						0.52		
Kennon and co-workers[54]	1937		6					52-68		0.1-3.9	0.0-1.90	25-40	0.6-7.0
Napier and Majumdar[45]	1938	Calcutta	25	20-40	M		7,111						
Osgood and co-workers[7]	1939	Portland	41	15-18	M		8,220	43.30	1.00	2.30	0.80	44.10	4.20
			87	15-18	M & F		8,190	46.30	1.00	2.10	0.70	42.20	4.30
Osgood and co-workers[8]	1939	Portland	198	19-38	M		7,300	53.20	0.60	2.00	0.50	38.80	4.20
			269	19-38	M & F		7,350	53.90	0.70	1.80	0.50	38.10	4.20
Kemp and Calhoun[55]	1901					778,000-862,000*							
Dameshek[58]	1932	Boston			M	500,000-900,000*							
						716,000							
Cumings[59]	1933	London	11			590,000-760,000*							
Olef[9]	1935	Boston	22		M	525,000*							
Buckman and Hallisey[56]	1921					284,000†							
Casey and Helmer[57]	1929					536,000†							
Present study	1939	Honolulu	137	16-25	M	253,000†	7,067	54.63		2.93	0.57	36.81	5.08

*Indirect method of platelet enumeration.
†Direct method of platelet enumeration.

values for different geographical regions may vary due to operation of factors of climate and altitude. Age differences for adult men, however, are thought not to occur, and if they do they may be so slight as to be insignificant. The extensive data collected in this investigation were analyzed to determine whether age or racial differences occurred for this series of persons. If age and racial differences were found not to occur, mean values could be calculated for all the blood elements for the entire group, these values to serve as standard blood values for the Hawaiian Islands for comparison with blood values reported for other geographical regions of the world.

The persons examined in this study ranged in age from 16 to 25 years, but the number in each age group was great enough to permit critical analysis only for the ages from 18 to 22 inclusive. General observations of the blood values for these age groups, supported by statistical analysis, showed that significant differences in the blood values did not occur. The values for the various blood elements for the different ages are summarized in Table III.

In the study of racial differences in blood values, sufficiently large numbers of persons to permit statistical analysis occurred only for the Caucasian, Chinese, and Japanese racial groups. The number of persons of other racial groups attending the University of Hawaii is so small that many years would be required for critical studies. For the three larger racial groups, statistically significant differences for none of the blood elements occurred. The values for the various blood elements for the different racial groups are summarized in Table IV.

Since significant age and racial differences for the various blood elements did not occur, all these data were combined for determination of normal blood values for young men residing in the Hawaiian Islands. The mean values, statistical constants, and the range of the values for the different blood elements are presented in Table V. The frequency of occurrence of the various blood values is graphically presented in histograms in Chart 1.

The mean values for the various blood elements obtained in this study for healthy young men of the Hawaiian Islands may be compared to those for men of other geographical regions summarized in Tables I and II. Such a comparison indicates that the values obtained in this study are closely similar to those given by the greater number of investigators. It is evident that particular or peculiar types of normal blood values do not exist for adult men of the Hawaiian Islands.

DISCUSSION

It is generally agreed that blood values for healthy adult males are constant. This feature of blood values has been pointed out by Osgood,[6] Andresen and Mugrage,[3] Wintrobe,[12] and many others. Our conclusion that age differences did not occur for the blood values of the series of healthy persons examined in this study was, therefore, anticipated, particularly in view of the narrow age limits of the group examined. Also, it has been pointed out by Osgood,[6] Mugrage and Andresen,[4] Sachs, Levine, and Griffith,[13] Goldhamer and Fritzell,[14] and many others that adult blood values are attained by males between the ages of 15 and 17 years. Our data tend to support these observations.

TABLE III

COMPARISON OF AVERAGE BLOOD VALUES OF DIFFERENT AGE GROUPS

AGE	NUMBER OF SUBJECTS	AVERAGE HB. (GM. PER 100 C.C. BLOOD)	AVERAGE NUMBER ERYTHROCYTES (MILLIONS PER C.MM. BLOOD)	AVERAGE VOLUME PACKED R.B.C. (C.C. PER 100 C.C. BLOOD*)	MEAN CORPUSCULAR VOLUME (C. MICRONS)	MEAN CORPUSCULAR HB. (MICROMICROGRAMS)	MEAN CORPUSCULAR HB. CONCENTRATION (PER CENT)
16	1	13.01	4,540,000	41.7	91.4	28.3	31.2
17	4	15.51	5,390,000	43.8	82.7	28.6	35.0
18	13	14.95	5,009,000	42.0	84.9	29.7	35.2
19	35	15.22	5,139,000	44.9	88.2	29.7	34.2
20	34	15.09	4,997,000	44.4	87.6	30.0	35.0
21	23	14.92	5,057,000	43.7	86.1	29.3	34.7
22	13	14.74	5,201,000	44.5	85.5	28.6	33.7
23	7	15.22	5,187,000	43.9	84.6	29.2	34.5
24	3	15.65	5,153,000	45.4	83.3	29.5	36.3
25	4	15.92	4,936,000	44.7	88.3	30.7	35.5
16-25	137	15.10	5,082,000	44.2	86.5	29.2	34.7

*Cell volume and corpuscular constants determined for 107 subjects.

AGE	NUMBER OF SUBJECTS	AVERAGE NUMBER PLATELETS (PER C.MM. BLOOD**)	AVERAGE NUMBER LEUCOCYTES (PER C.MM. BLOOD)	AVERAGE DIFFERENTIAL LEUCOCYTE COUNTS (PER CENT)				
				NEUTROPHILES	EOSINOPHILES	BASOPHILES	LYMPHOCYTES	MONOCYTES
16	1	245,000	7,550	60.00	1.34	0.67	33.67	4.00
17	4	252,000	6,600	55.42	4.17	0.42	34.08	5.84
18	13	253,000	7,270	51.31	3.64	0.74	38.72	5.59
19	35	261,000	6,700	54.65	2.90	0.57	36.55	5.48
20	34	256,000	7,250	55.42	2.57	0.49	36.62	4.70
21	23	248,000	6,820	56.32	3.11	0.52	34.97	5.06
22	13	251,000	7,450	57.51	2.81	0.70	34.08	4.97
23	7	233,000	6,090	52.09	2.53	0.76	40.48	4.34
24	3	239,000	6,463	52.22	2.45	0.78	39.89	4.45
25	3	269,000	6,140	50.00	5.34	0.34	40.34	4.00
16-25	136	253,000	7,067	54.63	2.93	0.57	36.81	5.08

†Platelets determined for 137 subjects.

TABLE IV

COMPARISON OF AVERAGE BLOOD VALUES OF DIFFERENT RACIAL GROUPS

RACE	NUMBER OF SUBJECTS	AVERAGE HB. (GM. PER 100 C.C. BLOOD)	AVERAGE NUMBER ERYTHROCYTES (MILLIONS PER C.MM. BLOOD)	AVERAGE VOLUME OF PACKED R.B.C. (C.C. PER 100 C.C. BLOOD*)	MEAN CORPUSCULAR VOLUME (C. MICRONS)	MEAN CORPUSCULAR HB. (MICROMICROGRAMS)	MEAN CORPUSCULAR HB. CONCENTRATION (PER CENT)
Caucasian	40	15.04	5,027,000	42.0	84.5	29.5	35.3
Chinese	39	15.10	5,158,000	45.1	87.0	28.4	33.9
Japanese	39	15.23	5,084,000	44.9	88.3	29.6	34.8
Korean	10	15.30	5,008,000	44.5	88.5	30.3	34.5
Caucasian-Hawaiian	8	14.62	5,049,000	43.3	85.4	28.8	34.6
Chinese-Hawaiian	1	16.60	5,110,000	44.6	87.1	32.3	37.2
All individuals	137	15.10	5,082,000	44.2	86.5	29.2	34.7

*Cell volume and corpuscular constants determined for 107 subjects.

RACE	NUMBER OF SUBJECTS	AVERAGE NUMBER PLATELETS (PER C.MM. BLOOD†)	AVERAGE NUMBER LEUCOCYTES (PER C.MM. BLOOD)	AVERAGE DIFFERENTIAL LEUCOCYTE COUNT (PER CENT)				
				NEUTROPHILES	EOSINOPHILES	LYMPHOCYTES	BASOPHILES	MONOCYTES
Caucasian	39	262,000	7,270	53.90	3.53	37.57	0.68	5.22
Chinese	39	247,000	7,090	53.97	2.57	37.69	0.52	5.12
Japanese	39	248,000	7,120	55.01	3.08	35.53	0.53	5.08
Korean	10	236,000	6,350	59.60	1.50	33.94	0.63	4.37
Caucasian-Hawaiian	8	293,000	6,620	52.88	2.71	39.29	0.42	5.00
Chinese-Hawaiian	1	233,000	7,000	58.67	2.34	32.34	0.00	6.34
All individuals	136	253,000	7,067	54.63	2.93	36.81	0.57	5.08

†Platelets determined for 137 subjects.

It has frequently been suggested that racial differences for blood values may occur and that these variations may account for differences in mean blood values reported by different authors. It is, however, a problem on which very little direct work has been undertaken. Hamre and Wong,[15] in a study of normal blood values for pre-school age children of different races, particularly Chinese, Japanese, and Caucasian, found the blood values to be similar, with

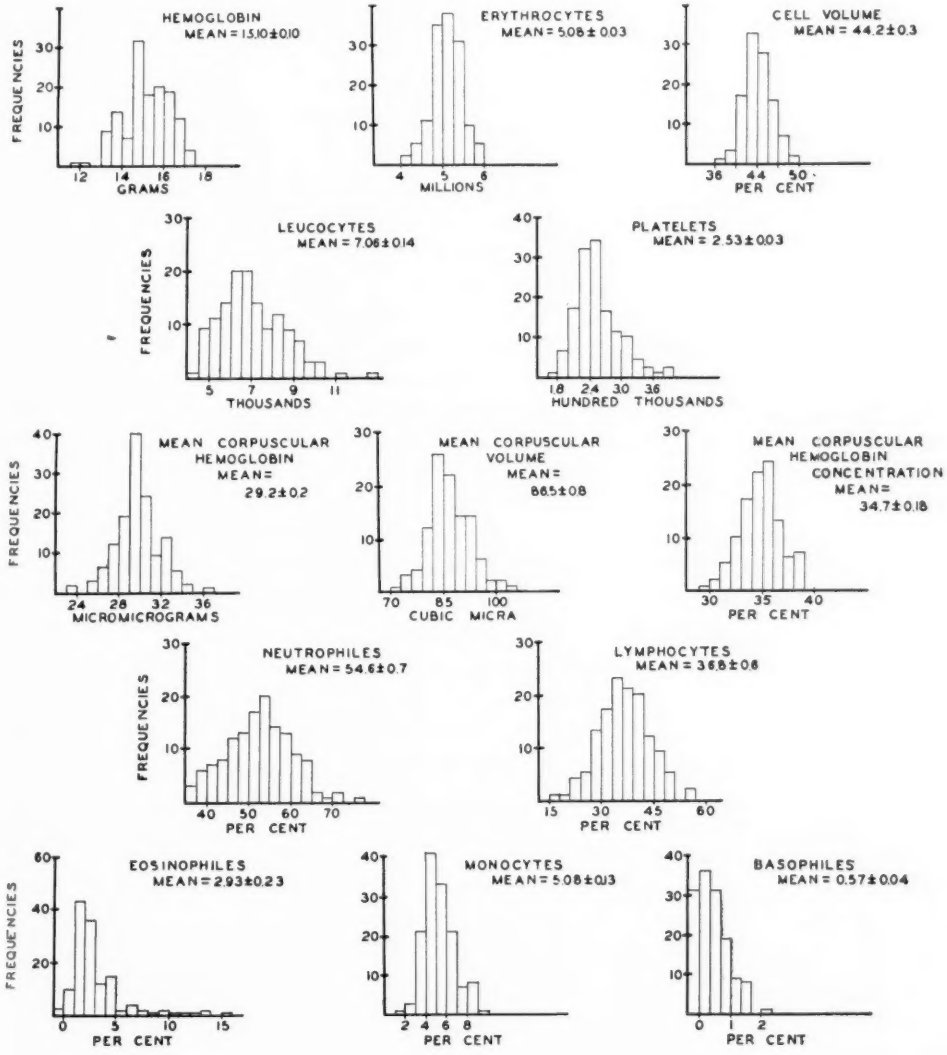

Chart 1.

no significant differences. Nelson and Stoker,[2] in studies of hemoglobin and red blood cell values of white and negro adult men, found no racial differences to exist. Myers and Eddy,[1] after a study of hemoglobin values reported in the literature for different races, came to the conclusion that racial differences did not occur. A study of Tables I and II, which include blood values for several races, indicates that, when due consideration is given to variations in technique

employed in the studies, racial differences in blood values do not exist, or if they exist, they are very small. The present study supports these conclusions, since it shows that for Chinese, Japanese, and Caucasian men of similar ages and environment, statistically significant differences for the mean values of none of the blood elements occur.

It has also frequently been suggested that the differences in mean blood values reported by different investigators may be due to geographical factors other than that of altitude. Walters,[16, 17] Wintrobe,[12] and Myers and Eddy[1] have concluded that significant differences of blood values for different geographical regions do not exist, and that blood values are generally the same for all regions of the world. The mean values reported by various investigators from various sections of the world, presented in Tables I and II, indicate that this conclusion must be accepted, and that the normal blood values for men residing in the Hawaiian Islands cannot be considered to differ from the blood values of men residing in other regions of the world.

Mean blood values for normal persons, as Nelson[18] and others have pointed out, represent only the central values of normal, the normal values ranging above and below these means. Mean values, however, may be employed in making comparisons for the degree of similarity and agreement of the results of different investigations. The mean values for hemoglobin, obtained by different investigators and presented in Table I, vary greatly though the greater number fall in the region of 14.5 to 15.5 Gm. per 100 c.c. of blood. Since various methods were used in making the hemoglobin determinations, it is possible that the variation in mean values obtained may be due to a great extent to the differences in methods employed. Where the technique and apparatus employed have been quite uniform, as for instance in the enumeration of red blood cells, the results of the different investigations are more nearly alike. The greater number of red blood cell values summarized in Table I fall into the interval of 5 to 5.5 millions per cubic millimeter of blood. Our mean hemoglobin value of 15.10 Gm. and mean number of red blood cells of 5.08 millions, therefore, fall in the regions which include the greater number of mean values reported for these blood elements.

The mean values for the volume of packed red blood cells of healthy adult men reported in the literature vary markedly. This is undoubtedly due to a great extent to differences in technique employed in the determinations. Other factors may also contribute to this difference in reported values. A number of recent workers (Walters,[17] Andresen and Mugrage,[3] and Mugrage and Andresen[4]) have reported mean values of 48 and 49 per cent for cell volume. These values are exceptional, the greater number of investigators having reported values ranging from 44 to 46 per cent. Our mean values of 44.2 per cent for the volume of packed red blood cells, therefore, fall in the group of more frequently reported values.

The corpuscular constants have received only little consideration except in recent investigations. The mean values for mean corpuscular hemoglobin concentration tend to be nearly alike and fall in the interval 32 to 35 per cent. Values for mean corpuscular hemoglobin are also in general agreement, and the greater number of mean values vary from 29 to 31 micromicrograms. Greater differences for mean corpuscular volume reported in the literature

occur and vary from 84 to 96 cubic microns. The same investigators who report high mean values for packed red blood cell volume have also reported high values for mean corpuscular volume. Our values of 34.7 per cent for mean corpuscular hemoglobin concentration, 29.2 micromicrograms for mean corpuscular hemoglobin, and 86.5 cubic microns for mean corpuscular volume agree with the values reported by the greater number of investigators and with the values of 35 per cent, 29.5 micromicrograms, and 87 cubic microns originally suggested by Wintrobe.[11]

TABLE V

MEAN VALUES, STATISTICAL CONSTANTS, AND RANGE OF VALUES FOR ENTIRE GROUP*

BLOOD ELEMENT	MEAN VALUE	STANDARD ERROR OF THE MEAN	STANDARD DEVIATION	RANGE
Hemoglobin in grams per 100 c.c. of blood	15.10	0.10	1.11	11.83 to 17.20
Erythrocytes in millions per cubic millimeter of blood	5.08	0.03	0.35	4.23 to 5.97
Volume of packed red blood cells in cubic centimeters per 100 c.c. of blood	44.2	0.3	2.8	37.5 to 51.6
Mean corpuscular volume in cubic microns	86.5	0.8	7.9	71.2 to 103.2
Mean corpuscular hemoglobin in micromicrograms	29.2	0.2	2.2	23.1 to 36.1
Mean corpuscular hemoglobin concentration in per cent	34.7	0.18	1.7	29.9 to 38.9
Platelets in ten thousands per cubic millimeter of blood	25.3	0.03	0.40	17.8 to 39.8
Leucocytes in thousands per cubic millimeter of blood	7.06	0.14	1.56	4.4 to 12.6
Differential leucocyte count in per cent				
Neutrophiles	54.6	0.70	8.0	38.6 to 78.3
Eosinophiles	2.93	0.23	2.7	0.0 to 15.6
Basophiles	0.57	0.04	0.48	0.0 to 2.3
Lymphocytes	36.81	0.6	6.9	16.3 to 55.6
Monocytes	5.08	0.13	1.47	1.6 to 9.3

*All mean values determined for 137 persons, except for leucocytes and differential leucocyte counts, which are for 136 persons, and cell volume and corpuscular constants for 107 persons.

The mean values reported for platelets may be divided into two groups, those obtained by the direct method and those obtained by the indirect method of enumeration. The mean values obtained by the two methods have been reviewed by Olef.[9] In general, the values obtained by the first method are about half as great as those obtained by the second method, and range from 250,000 to 300,000 per cubic millimeter of blood. We chose the direct method of enumerating platelets, and our mean value of 253,000 per cubic millimeter of blood compares well with the mean values obtained by this method by other investigators.

The mean values for the normal number of white blood cells reported in the literature available to us, and summarized in Table II, vary from 5,618 to 8,350 per cubic millimeter of blood. Our mean value of 7,067 falls well within the limits of these observed values. The mean values of 54.6 per cent for neutrophiles, 2.93 per cent for eosinophiles, 0.57 per cent for basophiles, 36.81 per cent for lymphocytes, and 5.08 per cent for monocytes obtained in this study compare closely to the mean values obtained by Osgood,[6] and Osgood

and co-workers.[7, 8] They, however, differ from the values usually given in textbooks in that the mean values for neutrophiles are lower while the mean values for lymphocytes are higher than values commonly considered to be normal.

SUMMARY

Data are presented for the blood values of 137 healthy young men residing in the Hawaiian Islands, and ranging in age from 16 to 25 years. They were of several different races, the larger number being Caucasians, Chinese, and Japanese. In this study, age or race differences were not found to occur for values for any of the various blood elements. The mean blood values obtained compared closely with those given by investigators in other parts of the world, and we have concluded that blood values for men in the Hawaiian Islands do not differ significantly from those of men residing elsewhere. The mean values, statistical constants, and range of observed values for the various blood elements are summarized in Table V.

REFERENCES

1. Myers, V. C., and Eddy, H. M.: The Hemoglobin Content of Human Blood, J. Lab. & Clin. Med. **24**: 502, 1939.
2. Nelson, C. F., and Stoker, R.: The Hemoglobin Concentrations and Erythrocyte Counts of Healthy Men, Folia haemat. **58**: 333, 1937.
3. Andresen, M. I., and Mugrage, E. R.: Red Blood Cell Values for Normal Men and Women, Arch. Int. Med. **58**: 136, 1936.
4. Mugrage, E. R., and Andresen, M. I.: Red Blood Cell Values in Adolescence, Am. J. Dis. Child. **56**: 997, 1938.
5. Garrey, W. E., and Bryan, W. R.: Variations in White Blood Cell Count, Physiol. Rev. **15**: 597, 1935.
6. Osgood, E. E.: Normal Hematologic Standards, Arch. Int. Med. **56**: 849, 1935.
7. Osgood, E. E., Baker, R. L., Brownlee, I. E., Osgood, M. W., Ellis, D. M., and Cohn, W.: Total, Differential and Absolute Leucocyte Counts and Sedimentation Rates of Healthy Adolescents Fifteen to Eighteen Years of Age, J. Lab. & Clin. Med. **24**: 905, 1939.
8. Osgood, E. E., Brownlee, I. E., Osgood, M. W., Ellis, D. M., and Cohn, W.: Total Differential and Absolute Leucocyte Counts and Sedimentation Rates Determined for Healthy Persons Nineteen Years of Age and Over, Arch. Int. Med. **64**: 105, 1939.
9. Olef, I.: The Enumeration of Blood Platelets, J. Lab. & Clin. Med. **20**: 416, 1935.
10. Hamre, C. J.: The Capillary Hematocrit Method of Determining Blood Cell Volume, J. Lab. & Clin. Med. **25**: 547, 1940.
11. Wintrobe, M. M.: The Size and Hemoglobin Content of the Erythrocyte, J. Lab. & Clin. Med. **17**: 899, 1932.
12. Wintrobe, M. M.: Blood of Normal Men and Women, Bull. Johns Hopkins Hosp. **53**: 118, 1933.
13. Sachs, A., Levine, V. E., and Griffith, W. O.: Copper and Iron in Human Blood, V. Normal Adolescent Children From 14 to 19 Years of Age, Arch. Int. Med. **60**: 982, 1937.
14. Goldhamer, S. M., and Fritzell, A. L.: A Study of the Red Blood Cell Count and Hemoglobin in the Adolescent Male, J. Lab. & Clin. Med. **19**: 172, 1933.
15. Hamre, C. J., and Wong, K. K. L.: Hematologic Values for Normal Children Three, Four, and Five Years of Age Living in Hawaii, Am. J. Dis. Child. **60**: 22, 1940.
16. Walters, O. S.: Normal Erythrocyte, Hemoglobin and Packed Cell Volume Standards in Young Men, J. Lab. & Clin. Med. **19**: 851, 1934.
17. Walters, O. S.: The Variation of Erythrocytes, Hemoglobin and Packed Cell Volume in Immediately Consecutive Samples of Venous Blood, Am. J. Physiol. **110**: 37, 1934.
18. Nelson, C. F.: The Range of Hemoglobin Concentrations and Erythrocyte Counts in Healthy Men and Women, Am. J. Clin. Path. **7**: 285, 1937.
19. Haden, R. L.: The Normal Hemoglobin Standard, J. A. M. A. **79**: 1496, 1922.
20. Sackett, G. E.: Hemoglobin and Iron in Blood, J. Lab. & Clin. Med. **10**: 1018, 1925.
21. Osgood, E. E.: Hemoglobin, Color Index, Saturation Index and Volume Index Standards, Arch. Int. Med. **37**: 685, 1926.
22. Wintrobe, M. M., and Miller, M. W.: Normal Blood determinations in the South, Arch. Int. Med. **43**: 96, 1929.
23. Murphy, W. P., Lynch, R., and Howard, I. M.: The Value of Determinations of the Iron Content of Whole Blood, Arch. Int. Med. **47**: 883, 1931.

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24. Foster, P. C., and Johnson, J. R.: Oxygen Capacity and Hemoglobin Content of Normal Blood of Men, Proc. Soc. Exper. Biol. & Med. **28:** 929, 1931.
25. Osgood, E. E., Haskins, H. D., and Trotman, F. E.: The Value of Accurately Determined Color, Volume and Saturation Indexes in Anemia, J. Lab. & Clin. Med. **17:** 859, 1932.
26. Haden, R. L.: Hemoglobin Standards, Am. J. Clin. Path. **3:** 85, 1933.
27. Sachs, A., Levine, V. E., and Appelsis, A.: Iron in Human Blood, Arch. Int. Med. **52:** 366, 1933.
28. Helmer, O. M., and Emerson, C. P.: The Iron Content of the Whole Blood of Normal Individuals, J. Biol. Chem. **104:** 157, 1934.
29. Broun, G. O., and Briggs, A. P.: Hemoglobin and Blood Cell Relations as Determined by Iron and Oxygen Capacity Methods, J. Lab. & Clin. Med. **19:** 886, 1934.
30. Sachs, A., Levine, V. E., and Fabian, A. A.: Copper and Iron in Human Blood. I. Normal Men and Women, Arch. Int. Med. **55:** 227, 1935.
31. Peterson, R. F., and Peterson, W. G.: The Differential Count at High Altitudes, J. Lab. & Clin. Med. **20:** 723, 1935.
32. Gram, H. C., and Norgaard, A.: Relations Between Hemoglobin, Cell Count and Cell Volume in the Venous Blood of Normal Human Subjects, Arch. Int. Med. **31:** 164, 1923.
33. Heilmeyer, L., and Hausold, I.: Die Hamoglobingehalt gesunder Männer und Frauen, eine Studie zur Definition des relativen, Masssystems der Hb-Bestimmung und zur Aufstellung des normalen Färbeindex, Deutsches Arch. f. klin. Med. **179:** 94, 1936.
34. Bierring, E., and Sørensen, G.: Haemoglobinbestemmelser med Van Slyke standardiserede Haemometre, Erythrocyttaellinger og Cellevolumbestimmelser paa Normale, med Orienterende Bemaerkninger vedrorende Indeksgeregning, Ugesk. f. laeger **98:** 822, 1936.
35. Dor, M.: Teneur du sang en hémoglobine chez l'adulte normal, Compt. rend. Soc. de biol. **172:** 1121, 1938.
36. Orias, O.: Teneur du sang en hémoglobine chez l'homme en argentine. Facteurs qui l'influencent, Comp. rend. Soc. de biol. **105:** 711, 1930.
37. Tenconi, J.: Relations entre le taux d l'hémoglobine, le volume et le nombre des hématies dans les anémies et les polyglobulies, Compt. rend. Soc. de biol. **108:** 133, 1931.
38. Wardlaw, H. S. H., Barry, H. C., McDonald, I. W., and McIntyre, A. K.: The Hemoglobin and Solids of the Blood of Australian Aborigines and Whites, Australian J. Exper. Biol. & M. Sc. **13:** 1, 1935.
39. Chamberlain, W. P.: The Red Blood Corpuscles and the Hemoglobin of Healthy Adult American Males Residing in the Philippines, Philippine J. Sc. **6:** 483, 1911.
40. Navarro, R. J.: Hematology in Filipinos. II. Normal Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration; The Various Normal Blood Indexes, J. Philippine Islands M. A. **17:** 611, 1937.
41. Sokhey, S. S., Gokhale, S. K., Malandkar, M. A., and Billimoria, H. S.: Red Cells, Hemoglobin, Colour Index, Saturation Index and Volume Index Standards. Part I. Normal Indian Men; A Study Based on the Examination of 121 Men, Indian J. M. Research **25:** 505, 1937.
42. Shourie, K. L., and Singh, N.: A Diet Survey of a Group of Sikh Soldiers, Indian J. M. Research **25:** 655, 1938.
43. Sankaran, G., and Rajagopal, K.: Hematological Investigations in South India. Part I. The Estimation of Hemoglobin, Indian J. M. Research **25:** 741, 1938.
44. Cropper, C. F. J., and Kahali, B. S.: Adult Haemoglobin Standards in Burma, Indian M. Gaz. **73:** 139, 1938.
45. Napier, L. E., and Majumdar, D. N.: Haematological Studies in Indians. Part IX. The Analysis of the Haematological Findings in 57 Cases of Anemia in Pregnant Tea-Garden Coolie Women, With Special Reference to the Results of Treatment, Indian J. M. Research **26:** 541, 1938.
46. Liknaitzky, I.: The Red Cell and Hemoglobin Content of the Blood in Young Male Adults Living on the Witwaterstrand, Quart. J. Exper. Physiol. **24:** 161, 1934.
47. Feinblatt, H. M.: Alimentary Leucocytosis in Eighty Normal Men: A Study in Reference to the Crise Hemoclasique of Widal, J. A. M. A. **80:** 613, 1923.
48. Stetson, R. P.: Normal Variations in White Blood Cells Under Conditions of Minimal Metabolism, Arch. Int. Med. **40:** 488, 1927.
49. Medlar, E. M.: The Extent of the Variations in the Leucocytes of Normal Count, Am. J. M. Sc. **177:** 72, 1929.
50. Stammers, A. D.: The Polymorphonuclear-Lymphocyte Ratio at an High Altitude of 5,750 Feet, J. Physiol. **78:** 335, 1933.
51. Schweizer, M.: The "Basal Level" of the White Cell Count in Man, Am. J. Physiol. **105:** 217, 1933.
52. Jones, E., Stephens, D. J., Todd, H., and Lawrence, J. S.: Studies in the Normal White Blood Cell Picture. I. Variations in Recumbent Basal Subjects and in Individuals With Change of Posture, Am. J. Physiol. **105:** 547, 1933.

53. Bryan, W. R., Chastain, L. L., and Garrey, W. E.: The Leucocyte Counts of Young Male Adults Observed After a Period of Rest and During Mild Activity in the Early Morning, Am. J. Physiol. **113**: 430, 1935.
54. Kennon, B. R., Shipp, M. E., and Hetherington, D. C.: A Study of the White Blood Cell Picture in Six Young Men, Am. J. Physiol. **118**: 690, 1937.
55. Kemp, G. T., and Calhoun, H.: Some New Observations on Blood Plates and Leucocytes, Am. J. Physiol. **5**: 4, 1901.
56. Buckman, T. E., and Hallisey, J. E.: Studies of the Properties of Blood Platelets, J. A. M. A. **76**: 427, 1921.
57. Casey, A. E., and Helmer, O. M.: Blood Platelet Counting, Proc. Soc. Exper. Biol. & Med. **27**: 655, 1930.
58. Dameshek, W.: A Method for the Simultaneous Enumeration of Blood Platelets and Reticulocytes, Arch. Int. Med. **50**: 579, 1932.
59. Cumings, J.: A Method for the Enumeration of Blood Platelets, Lancet **1**: 1230, 1933.

THE AGGLUTININ RESPONSE OF NORMAL PERSONS TO SKIN TESTS WITH BRUCELLERGEN AND BRUCELLA VACCINE*

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TWO types of antigens are commonly employed in skin testing for brucellosis. One, Huddleson's brucellergen, contains the purified protein nucleate fraction of Brucella cells. The other, Brucella vaccine, is a suspension of heat-killed organisms in physiologic saline, and is also used for therapy.

There is evidence that the intradermal injection of these substances stimulates the production of agglutinins. Giordano,[1] Goldstein,[2] Heathman,[3] and Meyer and Eddie,[4] have noted positive agglutination tests following skin tests with heat-killed organisms. Wide variations are reported, however, in the maximum titers obtained. For example, Goldstein recorded titers as high as 1:5,120, while the highest titer noted by Meyer was 1:80. Similar differences have occurred with brucellergen. Evans[5] found titers as high as 1:320, while Huddleson[6] states that "one skin injection of brucellergen may give rise to Brucella agglutinins in a low titer in a small percentage of individuals. The agglutinins disappear in about sixty days."

Among the factors contributing to these variations are (1) individual differences in agglutinin response, (2) the size of the skin test dose, (3) the nature of the antigen used for the agglutination tests, and (4) the technique of reading the agglutination tests. Since no single observer has studied both brucellergen and vaccine at the same time, it is difficult to draw conclusions as to their relative ability to stimulate agglutinin production.

Since this development of agglutinins may cause difficulty in interpreting agglutination tests, it is the routine procedure in some clinics to take blood for the agglutination test before the skin test is performed. However, since the results have been variable, as described, and since there is considerable difference of opinion concerning the relative merits of the skin and agglutination tests

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there is no general agreement on this point. For example, physicians using Huddleson's brucellergen are advised that "the first procedure to follow in arriving at a diagnosis is the performing of an intradermal test with brucellergen." Thus, in a clinic where patients are usually seen after consulting one or more other physicians, we are constantly faced with the problem of interpreting agglutination tests performed at intervals of several days to several weeks following skin tests, and it is of considerable importance to know as accurately as possible the effect of the skin test itself.

Due to marked local and systemic reactions in sensitive individuals, the size of the skin test dose has been reduced in the past few years, both for brucellergen and for the heat-killed organisms. Keller[2] has shown that the smaller doses of heat-killed organisms are equally as reliable as brucellergen for skin testing, and that reducing the size of the dose has abolished the more severe reactions previously noted.

Our purpose in undertaking this study was to determine the agglutinin response to commercial products commonly used at the present time, hoping we might, with these smaller doses, obtain information of value in interpreting agglutination tests performed on patients at varying intervals following skin tests. It also seemed of interest to compare the effects of vaccine and brucellergen, using the same antigen and the same technique in interpreting the tests.

METHOD AND MATERIALS

Fifty apparently normal, healthy adults, most of them members of the house staff of Stanford University Hospitals, volunteered for the study. All had negative Brucella agglutinations before being skin tested. One-half were given 0.1 c.c. of Huddleson's brucellergen 1:12,000, obtained from the Department of Bacteriology of the Michigan State College, and the other one-half were given 0.1 c.c. of the commercial Brucella vaccine, prepared by the Lederle Laboratories, diluted so that 0.1 c.c. contained forty million heat-killed organisms. The skin tests were read after forty-eight hours, an area of induration and edema of 5 mm. or more being considered positive. Agglutination tests were performed during the second and fourth weeks following the skin tests.

The antigen was prepared from *Br. abortus* strain 19, from which contaminants and rough variants were carefully excluded. A forty-eight-hour growth of the organisms was suspended in 0.5 per cent phenolized saline, and diluted to a density corresponding to approximately 4 cm. on the Gates apparatus. In performing the tube agglutination tests, serial dilutions of the serum, from 1:10 to 1:160, were added to constant amounts of the antigen. The tests were read after twelve hours' incubation at 37° C., and again after standing at room temperature for twenty-four hours. Tubes not showing a perfectly clear supernatant solution, with good clumping in the bottom, were regarded as negative. Tube agglutination tests were also performed on many sera with the stock antigen, prepared by the United States Department of Agriculture, as a check on the sensitivity of our own antigen. Finally, rapid slide agglutination tests were performed, using commercial *Br. abortus* febrile antigen, prepared by the Lederle Laboratories. The dilutions ranged from 1:20 to 1:160. These were read after standing ten minutes.

RESULTS

The results are presented in the accompanying tables. Individual skin and agglutination tests are recorded in Tables I and II, while Table III is a comparison of the results obtained with the two antigens. For convenience, Tables I and II are arranged in the following order: positive skin and agglutination tests, negative skin and positive agglutination tests, positive skin and negative agglutination tests, and negative skin and negative agglutination tests.

TABLE I

RESULTS IN PERSONS SKIN TESTED WITH BRUCELLERGEN

NUMBER	SKIN TEST	AGGLUTINATION TEST SECOND WEEK AFTER SKIN TEST		AGGLUTINATION TEST FOURTH WEEK AFTER SKIN TEST	
		SLIDE	TUBE	SLIDE	TUBE
1	+	0	1:10	0	1:10
2	+	1:40	1:40	1:20	1:20
3	+	1:80	1:40	1:40	1:40
4	+	0	1:20	1:20	1:20
5	+	1:20	1:20	1:20	1:20
6	+	0	0	0	1:20
7	+	1:20	1:40	1:40	1:40
8	+	0	1:10	1:20	1:20
9	+	1:80	1:80	1:40	1:40
10	-	1:20	1:40	1:40	1:40
11	-	1:80	1:80	1:40	1:80
12	-	1:40	1:40	1:40	1:40
13	-	0	1:20	0	1:20
14-15	+	0	0	0	0
16-25	-	0	0	0	0

TABLE II

RESULTS OF PERSONS SKIN TESTED WITH BRUCELLA VACCINE

NUMBER	SKIN TEST	AGGLUTINATION TEST SECOND WEEK AFTER SKIN TEST		AGGLUTINATION TEST FOURTH WEEK AFTER SKIN TEST	
		SLIDE	TUBE	SLIDE	TUBE
1	+	0	1:20	1:20	1:20
2	+	0	1:10	0	1:10
3	+	1:80	1:80	1:80	1:40
4	+	1:40	1:40	1:20	1:20
5	+	1:20	1:20	1:20	1:20
6	+	1:40	1:40	1:40	1:20
7	-	0	1:10	0	0
8	-	1:20	1:20	1:20	1:20
9	-	0	1:10	0	1:10
10	-	1:20	1:20	0	1:10
11	-	0	1:10	0	1:20
12	-	1:40	1:40	1:40	1:40
13	+	0	0	0	0
14-25	-	0	0	0	0

Eleven of the group tested with brucellergen and 7 of the vaccine group developed positive intradermal reactions. Five of the former and three of the latter developed red streaks up the arm, with swollen, painful axillary nodes. General malaise, chills, and fever occurred in two instances. In spite of these strongly positive reactions no local tissue necrosis resulted. A mild secondary infection of the skin developed in one instance. The local induration and edema

tended to be firmer and deeper following vaccine than following brucellergen, and persisted almost a month in two cases.

In each group about one-half of the persons failed to develop agglutinins. In the others the titers were uniformly low. The maximum of 1:80 was attained in only four instances. All but three with positive skin tests developed agglutinins. Of those with negative intradermal reactions, four in the brucellergen and six in the vaccine group also developed agglutinins, and the titers were equally as high as in those in whom the skin test was positive. From Table III it is apparent that there was very little, if any, difference in the response to the two skin test antigens.

TABLE III

RESULTS GROUPED ACCORDING TO THE MAXIMUM TITERS ATTAINED

SKIN TEST ANTIGEN	NUMBER OF POSITIVE SKIN TESTS	0	1:10	1:20	1:40	1:80
Brucellergen	11	11	2	5	4	3
Brucella-Vaccine	7	13	3	5	3	1

DISCUSSION

The high incidence (36 per cent) of positive intradermal reactions found in this study cannot, of course, be considered representative of the population as a whole, since only 50 persons were skin tested. However, it does serve to emphasize the point that healthy, active adults, who have never had symptoms of brucellosis, may have strongly positive skin tests. It does not seem presumptuous to state that several of these persons, if possessed with mild neurasthenic symptoms, would be regarded in certain circles as cases of chronic brucellosis. With the present enthusiasm for this disease, there is a tendency to forget that more than a positive skin test is necessary to make a diagnosis of active Brucella infection.

The agglutinin titers were consistently low. In one-half of the group none developed, and only a few were as high as 1:80. There was no apparent correlation between positive skin tests and agglutinin production, for a significant number developed agglutinins following negative skin tests, and the titers were as high as in those in whom the intradermal reaction was positive. From a comparative standpoint it can be said that the response to the two skin test antigens is almost identical.

From these results it would appear that titers above 1:80 can be regarded as due to contact with Brucella other than that of the skin test. Since only titers above 1:80 are usually regarded as indicative of active infection, the effect of the skin test can be disregarded entirely. Using a carefully prepared antigen and a standardized technique for reading the agglutination tests, we feel considerable confidence in this point of view.

As has been reported by others,[6] the titers obtained with the rapid slide test and with the macroscopic tube test were in close agreement. For many purposes the slide test, which is very simple to perform, would seem a satisfactory

diagnostic method. The possibility that our own tube antigen is relatively insensitive is precluded by the close agreement in agglutination titers obtained with the rapid slide method and with the stock antigen of the United States Department of Agriculture.

Several patients with low-grade fevers have reacted similarly to the "normal" persons described above. However, in two patients with acute febrile illnesses, both of whom had temperatures of above 40° C., agglutinin titers of 1:320 developed two weeks following skin tests. The intradermal reaction was positive in one and negative in the other. This excess rise in agglutinin titers, associated with fever, is well known and must be borne in mind in the interpretation of all agglutination tests.

The patients that present the greatest diagnostic problems are those with little or no fever, but with symptoms suggestive of chronic brucellosis. We can say with considerable assurance that in them the skin test will not influence the interpretation of the agglutination test. Where the blood culture is negative, and a careful correlation of the other laboratory procedures with the clinical picture is essential, it is of considerable practical aid to have such a criterion in dealing with patients who have been previously skin tested.

SUMMARY AND CONCLUSIONS

1. The agglutinin response of apparently healthy adults to the two commonly used types of Brucella skin-testing antigens has been studied, since this is a possible source of confusion in the clinical interpretation of the agglutination test. Commercial products, Lederle's Brucella vaccine and Huddleson's brucellergen, were used for the skin tests, to make the results of more practical value.

2. Positive intradermal reactions were present in 36 per cent of the 50 persons studied.

3. Approximately one-half of those skin tested failed to develop agglutinins, and in the others the titers were low, the maximum being 1:80.

4. There was little, if any, difference in response to the two antigens, and there was no correlation between positive skin tests and agglutinin production.

5. The practical significance of these results is discussed, and it is suggested that, except when high fevers are present, the agglutinin response to Brucella skin tests need not cause confusion in the interpretation of the agglutination tests.

REFERENCES

1. Giordano, A. S.: Brucella Abortus Infection in Man. The Intradermal Reaction as an Aid in Diagnosis, J. A. M. A. **93**: 1957, 1929.
2. Goldstein, J. D.: Cutaneous Reactions in the Diagnosis of Undulant Fever, J. Clin. Investigation **13**: 209, 1934.
3. Heathman, L. S.: A Survey of Workers in Packing Plants for Evidence of Brucella Infection, J. Infect. Dis. **55**: 243, 1934.
4. Meyer, K. F., and Eddie, B.: Laboratory Infections Due to Brucella, J. Infect. Dis. **68**: 24, 1941.
5. Evans, A. C.: Studies on Chronic Brucellosis. 2. Description of Techniques for Specific Tests, Pub. Health Rep. **52**: 9, 1937.
6. Huddleson, I. F.: Brucellosis in Man and Animals, London, 1939, The Commonwealth Fund.
7. Keller, A. E.: Comparison of Skin Reactions Obtained by Use of Brucella Nucleoprotein (Brucellergen) and of Heated Brucella Organisms in Sensitized Individuals, South. M. J. **33**: 1180, 1940.

OBSERVATIONS ON THE ELECTROCARDIOGRAM IN EPILEPSY AND COMPARISON WITH ELECTROCARDIOGRAM IN SEIZURES FOLLOWING CONVULSANT DRUG THERAPY*

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THE introduction of convulsive drugs into the treatment of mental disease has stimulated interest in the study of the physiologic changes associated with the seizures resulting from the use of these drugs, and also in the study of changes associated with convulsive seizures in general.

Bellet, Freed, and Dwyer[1] noted the electrocardiographic alterations during 58 shock treatments on 40 different patients during the hypoglycemic shock treatment of schizophrenia. In two-thirds of the shock treatments noteworthy electrocardiographic changes were obtained. These consisted of depression of the S-T segments, diminution of the height of the T wave, prolongation of the Q-T interval, auricular fibrillation, extrasystoles, shifting pacemaker, sinus arrhythmia, and sinoauricular heart block.

Orenstein[2] observed 15 schizophrenic patients treated with metrazol convulsions. Electrocardiographic changes varying according to the severity of the convulsions were noted following the convulsions. After a mild seizure there were a rapid rate, T-wave elevation, depression of S-T segment, and arrhythmia. After a severe convulsion he found in addition, sinus arrest, shifting of the pacemaker, auricular and ventricular premature contractions, coupling of beats, nodal escape, and rarely a marked bradycardia.

Messinger and Moros[3] studied the electrocardiographic findings in patients before and immediately after metrazol convulsions. In no instance did they find any significant deflection of the S-T interval. In 3 of 10 cases they found a slightly diminished amplitude of the T waves, and in 7 others a slightly increased amplitude of the T waves.

Levine, Piltz, and Reznikoff[4] also studied the effects on the electrocardiogram of metrazol shock therapy and found no significant changes. In one patient they found inversion of the T wave in Leads II and III after the seizures. Since they believed this to be due to anoxemia, they gave the patient 100 per cent oxygen by inhalation for fifteen minutes. Thirty minutes later an electrocardiogram was taken; it recorded that most of the T waves in Leads II and III were upright.

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Lennox and Cobb[5] obtained electrocardiographic records of patients during petit mal and myoclonic seizures, and in these instances neither the rhythm nor the amplitude of the waves was appreciably affected.

Erickson,[6] in a clinical study of changes in the circulatory system during or immediately preceding an attack of epilepsy, observed the electrocardiographic picture also. In most cases only one lead of the electrocardiogram was taken in order to avoid picking up too many electrical effects from the muscles during the convulsion. He used a direct chest lead, one electrode being placed over the manubrium sterni just below the sternal notch and the other just below the point at which the apex beat of the heart was palpated. He obtained satisfactory electrocardiographic tracings during 54 epileptic seizures of various types in 17 different patients. In none of the 54 attacks observed was there any dropping out of the QRS interval of the electrocardiogram. The T wave was unchanged in all but 4 patients. In 4 the T wave became biphasic or flattened at the end of the attacks.

Winternitz[7] observed a patient in status epilepticus, and for several days after the occurrence he noted changes in the electrocardiogram, a long R-T segment.

NEW DATA

The present study concerns mainly the electrocardiographic findings in a group of 20 epilepties observed at the Minnesota State Colony for Epilepties at Cambridge, Minn. Electrocardiograms were taken before seizures, during seizures, and at various intervals after seizures. Three standard leads and Lead IVF, as adopted by the American Heart Association, were taken on each patient. Thirty-eight electrocardiograms were taken in this series. The age of the patients varied from 13 years to 45 years. There was neither history nor clinical signs of heart disease in any of them.

Diminished amplitude of the T waves and increased depth of the T wave, where the T wave was normally inverted, were noted in practically every instance after the seizures. Other significant changes were observed in 5 of 12 patients in whom comparative electrocardiograms were made during seizures and in the period of remission. These changes consisted of variations of the electrical axis, changes in rhythm, various degrees of auriculoventricular block, dislocation of the pacemaker, and inversion of the T waves. Some of these changes were noted immediately after the seizures, and others for periods as long as four and one-half hours after a seizure. In one patient a tracing taken immediately after the termination of a convulsive seizure showed a negative T wave, whereas a positive T wave was noted immediately preceding the seizure. Another patient showed a change of electrical axis to right axis deviation, ventricular extrasystoles, and negative T waves in Leads I and IV after the seizure, and a normal electrocardiogram during the period of remission. A detailed description of the significant electrocardiographic findings is given in Table I.

A comparison of the electrocardiographic findings we observed following epileptic seizures, with those noted by other workers following convulsive seizures

induced by insulin shock and metrazol therapy of schizophrenia, reveals the fact that the changes are similar in character in each instance. This would lead us to believe that the physiologic changes preceding the convulsion and during the convulsive seizures are the same whether the convulsions are the result of insulin shock, metrazol, or epilepsy. Also in epileptic seizures there is an intrinsic factor which acts on the cerebral circulation and the cardiovascular circulation in general, in a manner similar to the extrinsic factors, insulin, and metrazol in their respective convulsive seizures.

TABLE I

NAME	AGE	SEX	TIME OF SEIZURE	DATE	TIME OF EKG	INTERVAL	EKG FINDINGS
F. J.	15	F	7 A.M. 8 A.M. 10:45 A.M.	12/11/39	1:15 P.M.	2 hr. 30 min.	T wave diminished in height
			None	1/ 8/40	10:25 A.M.		Negative
M. Sw.	38	F	9 A.M.	12/11/39	1:28 P.M.	4 hr. 28 min.	Slight T wave changes. Variations in height of R in Lead IV
			None	1/ 8/40	1:45 P.M.		Negative
G. U.	16	F	8 A.M. 8:30 A.M.	12/11/39	11:20 A.M.	2 hr. 50 min.	Ventricular extrasystoles: right axis deviation. Negative T wave in Leads I and IV
			None	1/ 8/40	11:30 A.M.		Negative
M. St.	19	F	6 A.M.-7:30 P.M. (28 seizures)	12/11/39	11:00 A.M.	None	Diminished height R and T waves. Wandering pacemaker. Negative T wave in Lead III during seizure
					1:50 P.M.		Increased height of R and T waves
			None	1/ 8/40	11:20 A.M.		Negative
L. F.	22	F	6 A.M.-8 P.M. (10 seizures)	12/11/39	11:40 A.M.	None	Diminished height R and T waves. Increased depth T wave during seizure
				1/ 8/40	11:10 A.M.		Negative
G. G.	21	F	2:50 P.M.	12/11/39	3:10 P.M.	20 min.	T wave changes. Ventricular extrasystoles
			None	1/ 8/40	1:50 P.M.		Negative
V. D.	45	M	11:00 A.M.	5/22/39	11:30 A.M.	30 min.	Delayed auriculoventricular conduction with variable P-R interval (ventricular escape)
			None	5/25/39			Negative

POSSIBLE CAUSES OF EKG CHANGES

Clinical observation and experimental research by many observers have shown that electrocardiographic changes similar to those observed following convulsive seizures are an indication of myocardial ischemia or anoxemia of the heart muscle.

Kountz and Gruber[8] produced anoxemia in animals, and when the oxygen saturation fell below 50 per cent, electrocardiographic changes similar to those observed clinically in coronary occlusion were found. Release of the animals from the anoxemic state caused the electrocardiogram to return to normal. They

also produced pictures similar to anoxemia by the injection of pitressin, the primary action of which is vasoconstriction. The electrocardiographic changes in these instances could be made to disappear by the use of vasodilators, such as sodium nitrite. Milles and Smith[9] produced similar changes by the intravenous injection of epinephrine. It may be assumed, therefore, that the electrocardiographic changes seen following convulsive seizures from insulin shock, metrazol, and epilepsy are due to myocardial ischemia or anoxemia of the heart muscle.

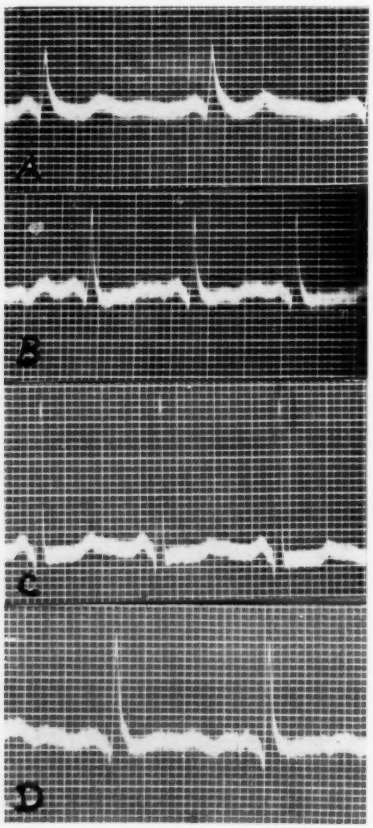

Fig. 1.

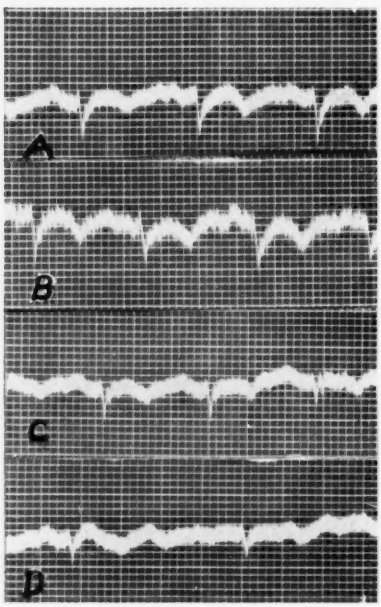

Fig. 2.

Fig. 1.—EKG Lead III showing T-wave changes after seizures. *A*, (12/11/39) 11:00 A.M. —one-half hour after seizure. *B*, 11:20 A.M. During seizure. *C*, 1:50 P.M. two and one-half hours after seizure. *D*, Control (1/8/40). No seizures.

Fig. 2.—EKG Lead III showing T-wave changes after seizures (12/11/39). *A*, 11:40 A.M. —one hour after seizure. *B*, 11:45 A.M. During seizure. *C*, 1:45 P.M.—two hours after seizure. *D*, Control (1/8/40). No seizures.

Bellet, Freed, and Dwyer[1] believe that in insulin shock therapy the lack of available carbohydrates and the increased work of the heart during hypoglycemic shock of long duration suggest that anoxemia may be a factor in producing the cardiac effects. They base their conclusions on previous investigations which have shown that lowering of the blood sugar reduces the oxygen consumption of the brain; also that the profound changes which occur in cerebral metabolism during hypoglycemia and the resultant coma may indirectly affect the heart.

Messinger and Moros[3] formulate an hypothesis to account for the diverse cardiovascular changes they noted in their study of the effects of insulin and metrazol in these cases. They are of the opinion that persons differ in their response to various types of stimuli, and particularly in the degree of their response to drugs of the sympathetic-adrenal type. It is now recognized that the prolonged administration of insulin (as in shock treatment) may induce an altered state of reactivity of the sympathetic system primarily in the direction of an increased sensitivity of response to adrenalin and sympathetic-mimetic

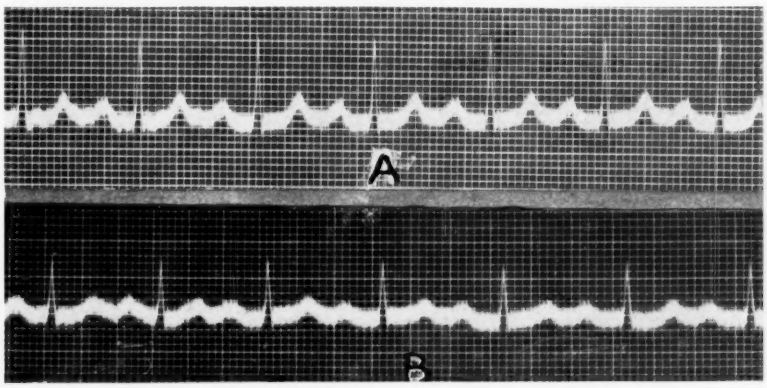

Fig. 3.—EKG showing delayed auriculo-ventricular conduction with variable P-R interval. *A*, Control (5/25/39). Normal tracing. *B*, Thirty minutes after seizure (5/22/39) showing delayed auriculoventricular conduction with variable P-R interval, simulating ventricular escape.

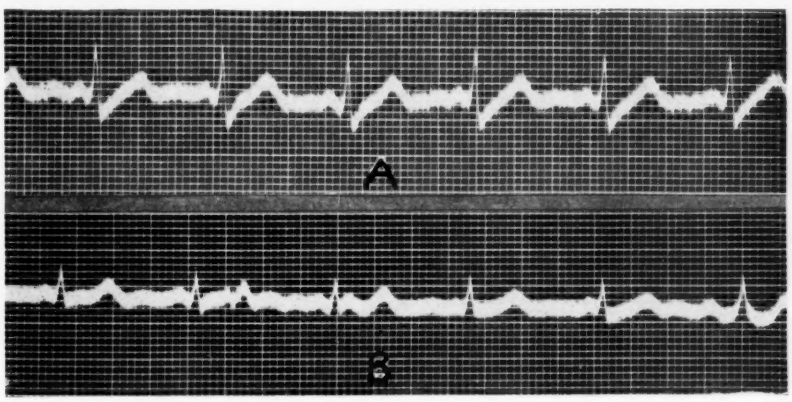

Fig. 4.—EKG showing wandering pacemaker after a seizure. *A*, Control (1/8/40). Normal tracing. *B*, After several seizures (12/11/39) showing variable P waves, indicating a wandering pacemaker.

drugs. Also metrazol may produce transitory effects which are typical of a mild hyperadrenalemia. That adrenalin may cause heart changes has been shown by the work of Milles and Smith[9] and others. These observers showed that electrocardiographic changes similar to those occurring in convulsive seizures could be produced by the intravenous injection of adrenalin.

Levine, Piltz, and Reznikoff,[4] contending that anoxemia might account for the electrocardiographic changes seen after metrazol therapy, gave their patient, who showed an inverted T_2 and T_3 after metrazol convulsions, 100 per cent

oxygen by inhalation for fifteen minutes. Thirty minutes later the electrocardiogram revealed that most of the T waves in Leads II and III were upright.

In the study of chemical and physiologic conditions, which may be contributing factors to epileptic seizures, four major conditions are usually given; namely, acid-base balance, fluid balance, oxygenation, and circulation. That these factors may be interrelated and responsible for cardiovascular changes in epilepsy, as depicted by the electrocardiogram, may be assumed from our observations on this group of patients which we studied.

Reduction of alkalinity and induction of acidosis, either by fasting, physical exercise, or the ketogenic diet, is known to precipitate seizures.

McBroom,[10] in collaboration with Hirschfelder and Haury,[11] made a study of blood potassium in epilepsy. They found a gradual increase in the potassium up to the time of seizure, followed by a drop immediately after the seizure.

Moglia[12] studied the effects of adrenalin on the potassium in the plasma. He injected adrenalin intravenously into chloralosed dogs at a constant rate for fifteen minutes. A dose of 5 gamma per kilogram per minute produced an initial increase of plasma potassium, lasting from five to ten minutes, followed by a prolonged decrease. A dose of 0.5 and 0.25 gamma produced a prolonged decrease without the initial increase. The larger doses were similar to the amounts secreted by the adrenals in states of emergency; the smaller ones were similar to the physiologic amounts of adrenalin and sympathin.

Thomson[13] studied the effect of potassium on the heart by giving potassium chloride and citrate in doses varying from 3 to 30 Gm. daily to 24 patients with various diseases, and observed the effects on the electrocardiogram. In 14 patients there was an increased height in the T waves in one or more leads after giving the potassium. The average increase in the level of the serum potassium was 8.3 mg. per 100 c.c., as compared with 2.6 mg. per 100 c.c. in the patients who showed no such change. In one patient the T wave became more diphasic. In two patients definite degrees of heart block were produced. In one there was prolongation of the P-R interval. Other changes also were noted. Thomson advances the hypothesis that these changes may be mediated through the action of the potassium ion on the myocardium because of the similarity of these changes to those produced by vagal stimulation, by acetylcholine, and by pitressin.

Changes in the cerebral circulation and also in the general circulation, probably resulting from this vasoconstrictor effect, have been shown by many observers. Penfield[14] reported the cessation of cerebral pulsations during convulsions occurring when the brain was exposed by craniotomy.

Olkon[15] studied the microscopic vascular changes in the brain, internal viscera, and skin of living animals in relation to convulsions. Following convulsions induced by the injection of excitant drugs, capillary changes were invariably found in the pia-arachnoid, skin, and internal organs. These consisted of twisting, irregularity, and deformity, and a sluggishness in the circulating cells within their lumen.

Denyssen and Watterson[16] also are of the opinion that a vasoconstrictor effect is responsible for the convulsions. They gave vasodepressor drugs, such as amyl nitrite, sodium nitrite, and histamin, prior to the administration of convulsive doses of metrazol. No fits occurred.

CONCLUSIONS

The electrocardiographic findings which we have observed during and after epileptic seizures tend to show that cardiovascular changes occur following epileptic seizures, and that these changes are part of circulatory changes throughout the body. As a result of this, and from the experimental and clinical observations of other workers, the supposition is made that the epileptic seizures are due to a cerebral anoxemia resulting from an ischemia of the brain following vasoconstriction. This is probably the result of an electrochemical change and adrenalin-like action on the cerebral vessels.

After comparing our findings with those of other workers who studied the electrocardiographic changes following seizures after convulsant drug therapy, we conclude that the physiologic changes during and after convulsive seizures from excitant drugs are the same as those due to epilepsy; also that the extrinsic factors, such as insulin shock and metrazol, probably produce a similar adrenalin-like reaction as the intrinsic factor which brings on the cerebral ischemia and anoxemia in epileptic seizures.

We wish to thank Dr. D. E. McBroom, Superintendent, Minnesota Colony for Epileptics, and his associates for the opportunity to observe the patients at the institution and for their valuable cooperation and assistance in making this study possible.

REFERENCES

1. Bellet, Samuel, Freed, Herbert, and Dwyer, W. W.: The Electrocardiogram During Insulin Shock Treatment of Schizophrenia and Other Psychoses, Am. J. M. Sc. **198**: 533, 1939.
2. Orenstein, Leo L.: Physiologic Observations Following Induced Convulsions, New York State J. Med. **39**: 1921, 1939.
3. Messinger, E., and Moros, N.: Cardiovascular Effects of Large Doses of Metrazol as Employed in the Treatment of Schizophrenia, Ann. Int. Med. **13**: 1184, 1940.
4. Levine, Harold, Piltz, George F., and Reznikoff, Leon: Electrocardiographic Studies in Metrazol Shock Therapy, Am. J. M. Sc. **199**: 201, 1940.
5. Lennox, William G., and Cobb, Stanley: Epilepsy From the Standpoint of Physiology and Treatment, Medicine **7**: 108, 1928.
6. Erickson, Theodore C.: Cardiac Activity During Epileptic Seizures, Arch. Neurol. & Psychiat. **41**: 511, 1939.
7. Winternitz, M.: Myokardnekrose nach Status epilepticus, Med. Klin. **29**: 1080, 1933.
8. Kountz, W. B., and Gruber, C. M.: Proc. Soc. Exper. Biol. & Med. **27**: 170, 1929.
9. Milles, M., and Smith, P. W.: Effects of Epinephrine on the Heart, Am. Heart J. **14**: 198, 1937.
10. McBroom, D. K.: Personal communication.
11. Hirschfelder, Arthur D., and Haury, Victor G.: Variations in Magnesium and Potassium Associated With Essential Epilepsy, Arch. Neurol. & Psychiat. **40**: 66, 1938.
12. Moglia, J. L.: The Effect of Adrenalin on Potassium in Plasma, Rev. Soc. argent. de biol. **15**: 5, 1939.
13. Thomson, W. A. R.: The Effect of Potassium on the Heart in Man, Brit. Heart J. **1**: 269, 1939.
14. Penfield, W.: The Evidence for a Cerebral Vascular Mechanism in Epilepsy, Ann. Int. Med. **7**: 303, 1933.
15. Olkon, D. M.: Epilepsy and the Convulsive State, Association for Research and Mental Disease, Baltimore, 1931, Williams & Wilkins Co., Vol. 7.
16. Denyssen, J. A. F., and Watterson, D. J.: On Mechanism of Cardiazol Convulsion, J. Ment. Sc. **84**: 1002, 1938.

PERIPHERAL NERVES IN CHRONIC ATROPHIC ARTHRITIS*

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THE obvious fact that chronic atrophic arthritis is not solely a joint disease, but one in which many systems of the body are involved, has caused us to undertake a more detailed investigation of the entire organism. We have begun with a re-examination of all the pathologic changes. Recently, the muscles and valves of the heart have been examined and noteworthy findings have been reported. Because of the nature of the pains, trophic conditions, vasomotor alterations, and heightened reflexes, a more detailed study of the peripheral nerves and sympathetic chains were undertaken. In earlier studies only the brains and spinal cords were examined; no specific or characteristic lesions were found.

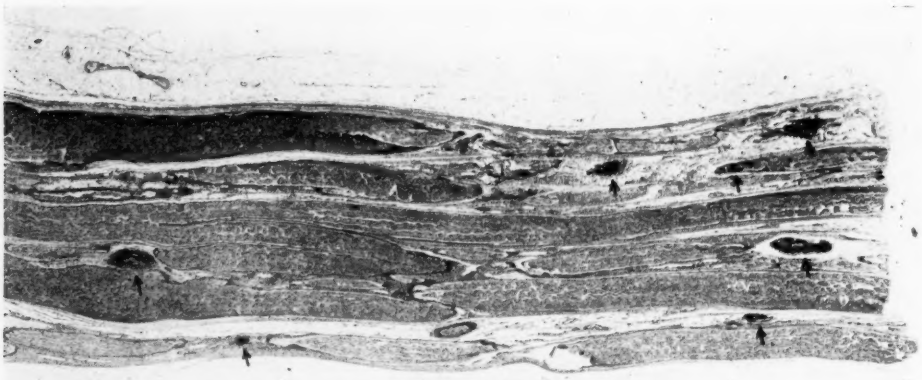

Fig. 1.—A longitudinally cut section through a femoral nerve of a patient with severe chronic atrophic arthritis, ×9. The arrows indicate the perineural or interfascicular location of the nodules.

In our present study, covering the past two and one-half years, hitherto undescribed pathologic changes involving the peripheral nervous system have been found. These are recorded here briefly; a more detailed report will follow.

The brains of 11 patients with definite chronic atrophic (rheumatoid) arthritis and the peripheral nerves in five of the patients were examined. There were no specific changes in the central nervous system; however, in two of five cases of chronic atrophic arthritis characteristic and severe pathologic lesions

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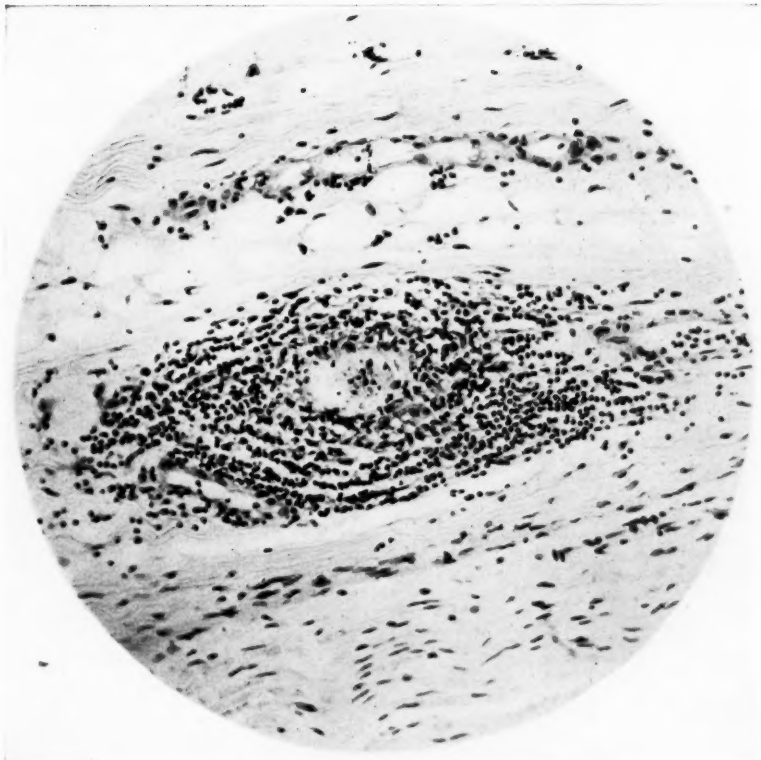
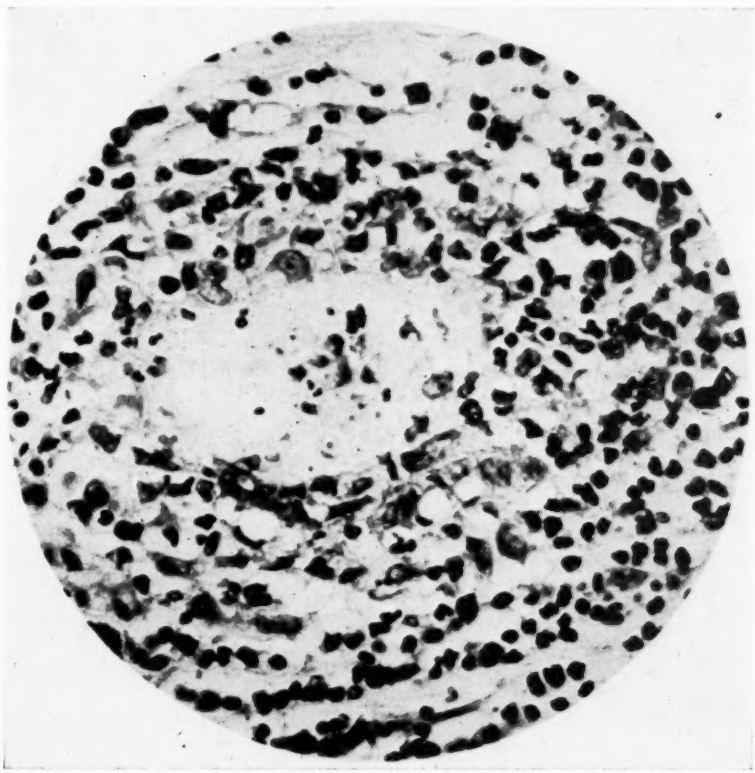


A.



B.

Fig. 2.—*A* and *B* represent the same nodule in two different magnifications, ×150 and ×550, respectively. Three distinct zones, the inner necrotic zone, the intermediate zone of proliferating mesenchymal cells, and the peripheral lymphocytic zone, are clearly shown. The intermediate zone in this nodule is somewhat smaller than usual.

were found in peripheral nerve trunks (sciatic, femoral, brachial plexus). In one case the lesion in the peripheral nerves was somewhat milder, and in the last two cases the changes were only slight. The characteristic findings were represented by sharply circumscribed nodules of round or oval shape. The nodules are not seen grossly in unstained specimens. However, they can be seen with the naked eye in stained slides. Fig. 1 represents a longitudinally cut part of a peripheral nerve magnified nine times to show the distribution of the nodules in a peripheral nerve. The nodules are located in the perineurium and are less common in the epineurium; they are never found in the endoneurium of the peripheral nerves. The size of a single nodule varies. Serial sections through a single nodule show an involvement 0.14 to 0.2 mm. in diameter. The nodules are often found in groups of three or four in the same longitudinally cut section. Histologically, each nodule consists of two or three zones: a central zone of necrosis which may or may not be present, an intermediate zone of proliferating mesenchymal cells, and a peripheral ringlike zone of inflammation with lymphocytes and plasma cells. The cells in the intermediate zone are of the so-called epithelioid type. They are polyhedral and have large elongated irregular nuclei, poor in chromatin. Clear cell outlines are usually not demonstrable. Fig. 2 *A* and *B* are photomicrographs, ×175 and ×550, respectively. The three zones of the nodule are clearly seen. Conglomeration into larger areas is never found. This finding has to be classified as "rheumarthritic nodulous perineuritis." The specificity of this change has been confirmed by examination of peripheral nerves from 86 control cases which were examined without knowledge of the clinical history or the findings at autopsy. Six cases showed pathologic changes in the peripheral nerves. In one case with the finding of perineuritic nodules the clinical history revealed that the patient had chronic atrophic arthritis for many years. Recently in continuing our control examination, a new case with perineuritic nodules in peripheral nerves was found. This was an 80-year-old female with old rheumatic heart disease (clinically diagnosed as rheumatoid arthritis). The pathologic changes in the five nonarthritic control cases (one of Buerger's disease, two of dermatomyositis, one of postencephalitic parkinsonism, one of carcinomatous infiltrations of the epineurium) differed distinctly from the perineuritic changes in chronic atrophic arthritis. We consider rheumarthritic nodulous perineuritis a specific active inflammatory process concomitant and parallel with other rheumatic pathologic features in chronic atrophic arthritis, such as rheumatic heart disease, subcutaneous nodules, and synovial inflammation and proliferation.

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BRUCELLOSIS: STUDIES EMPHASIZING STRAIN VARIATION IN SEROLOGIC TESTING*

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THE purpose of this study is to explain the wide variation in agglutination titer and opsonocytophagic activity which we have observed from various laboratories. In many cases the difference in results were too great to be accounted for as technical variations. To illustrate, a state laboratory reported a negative Brucella agglutination titer on the same serum which we were able to demonstrate a positive titer of 1:640 with three commercial antigens. In another instance, the laboratory using a single strain of the Brucella reported 69 per cent of polymorphonuclear neutrophiles showing marked phagocytosis. One week later we did not find marked phagocytosis in any of the cells. Therefore, we outlined a plan to investigate the agglutination titer and cytophagic activity when the tests were performed by the same technician with 20 different strains.

In this study we selected 34 patients from private practice. From all, blood serum had yielded positive agglutination tests either in our laboratory or in other laboratories. The antigens were prepared from 20 smooth strains of the Brucella obtained from the universities of Michigan, Minnesota, Illinois, Wisconsin, and the Public Health Laboratory in Washington. All strains were negative to thermoagglutination and dye agglutination before and after the experiment. All strains were injected into guinea pigs. Fourteen strains produced death, 8 of which showed gross Brucella lesions and 6 of which produced microscopic changes only. The other 6 showed evidence of disease by loss of weight, roughening of hair, comparatively low white blood cell counts and maintenance of high agglutinin titers. All strains were determined to react with known positive and negative bloods.

Opsonocytophagic Tests.—Suspensions were prepared from forty-eight-hour cultures of Brucella organisms in saline with pH 7.02 to 7.1. Turbidity was adjusted to McFarland nephelometer No. 25. Five cubic centimeters of blood were collected in sterile tubes that had been filled with the proper amount of citrate to give a final concentration of 0.8 per cent and marked to the level to which the blood should be added. All tests were completed within four hours after the blood was collected. Performance of the test was that described by Huddleson,[1] except that smears were made by the cover slip method since this method, in our opinion, is superior to the slide method in that less mechanical injury to the cells is induced when blood spreads by capillarity rather than by

*From the Hixon Laboratory for Medical Research, University of Kansas, by the Boylan Foundation.

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actual smearing. Smears were stained with Hasting's stain. One hundred cells were counted; the findings are, therefore, expressed as percentage of cells which showed marked, moderate, weak, and negative cytophagic activity.

Agglutination Test.—Strain antigens were prepared by washing a seventy-two-hour culture of Brucella from liver infusion slants with saline containing 0.5 per cent phenol. The suspensions were strained, centrifuged, and resuspended. The stock antigens were stored as concentrated suspensions. The dilution was adjusted to McFarland nephelometer No. 1.5 for each antigen, recorded, and made up as needed. Twenty cubic centimeters of patient's blood were collected in a chemically clean tube, rimmed, and centrifuged. The serum was removed and stored at 7° C. until the agglutination series could be run within the next two days.

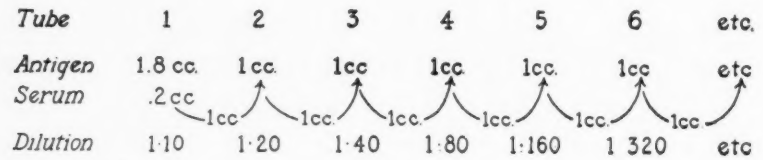

Fig. 1.

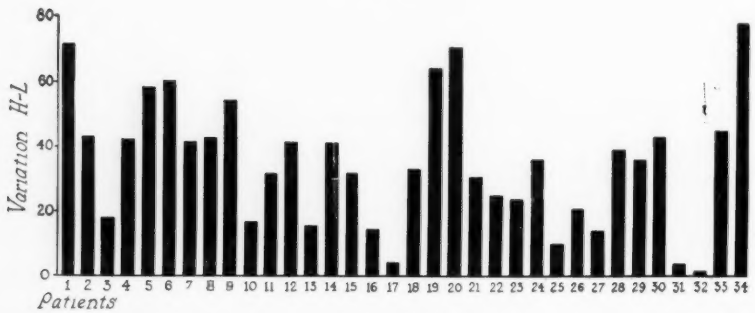

Chart 1.—Range of variation of opsonocytophagic index numbers, as determined by Foshay-LeBlanc method, in 34 patients with brucellosis when tested with 20 strains of Brucella.

To prevent numerous pipettings, the agglutination test was performed as shown in Fig. 1. The method is more accurate by reason of fewer operations in pipetting small amounts, and is a timesaver as well. The tests were incubated at 37° C. in a hot-air incubator for twelve hours and allowed to stand at refrigerator temperatures for twenty-four hours, after which readings were made. The titer was read from the last serum dilution showing complete agglutination, that is, the supernatant fluid completely clear with the bacteria completely sedimented in clumps.

EXPERIMENTAL RESULTS

Charts 1 and 2 record our results. Chart 1 represents the correlation of results of the opsonocytophagic tests calculated by the Foshay-LeBlanc method,[2] the final results being obtained by subtracting the minimum index from the maximum index to determine the variation when each serum was set up against 20 strains of Brucella simultaneously.

Chart 2 shows the correlation of agglutination titers demonstrating the variation in agglutinins by subtracting the minimum titer from the maximum titer in terms of number of tubes apart in the test set-up.

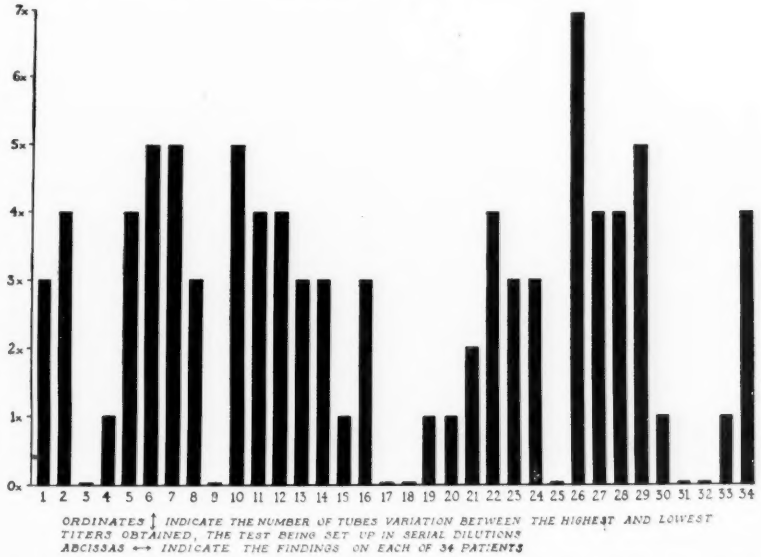
7x
6x
5x
4x
3x
2x
1x
0x
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
ORDINATES ↕ INDICATE THE NUMBER OF TUBES VARIATION BETWEEN THE HIGHEST AND LOWEST TITERS OBTAINED, THE TEST BEING SET UP IN SERIAL DILUTIONS
ABCISSAS ↔ INDICATE THE FINDINGS ON EACH OF 34 PATIENTS

Chart 2.—Variation in agglutinin titers of patients with brucellosis tested with antigens from 20 strains of Brucella.

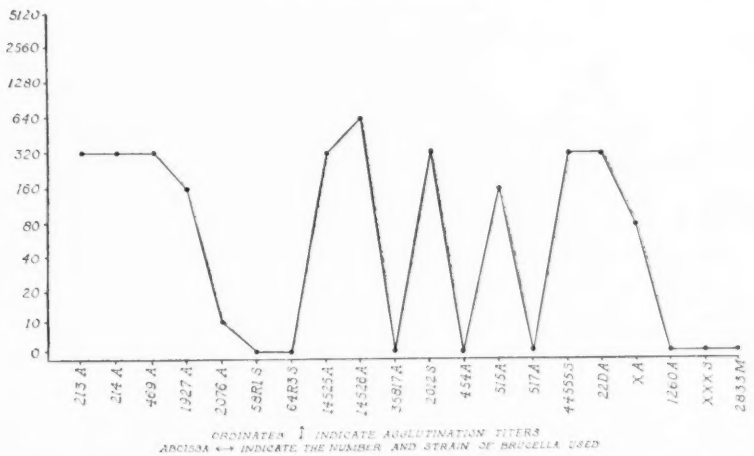

Chart 3.—Agglutination series on a single patient (EM-26), showing marked variation of agglutination titers when tested with 20 strains of Brucella.

It is observed from the studies that there is wide fluctuation in the majority of instances. When there was little variation, as in the patients 17, 31, 32 in Chart 1, their actual opsonocytophagic activity was almost negative; similarly, when the records of patients 3, 9, 17, 18, 25, 31, 32 in Chart 2 are examined, their agglutination titers were negative throughout. When there was only a difference of one tube, probably not outside the range of individual variation, the titers were either very low (1:10) or very high (1:2,560 to 1:5,120 and 1:1,280 to 1:2,560). In the analysis of Chart 2 a variation is noted in the study of the

agglutination reaction, the widest being from no agglutination to complete agglutination in a dilution of 640 in the case of EM-26. This is shown graphically in Chart 3. The range of agglutination is from 0 to 1:640. Ten strains were agglutinated in a dilution of 1:160 or above. Eight strains were not agglutinated.

DISCUSSION

It will be noted from the foregoing results that there is wide variation between the agglutination and opsonocytophagic activity of the same blood taken at the same time and tested under the same conditions with the 20 strains employed.

A like observation was made in 1937 by Campbell and Greenfield,[3] employing four strains of Brucella in agglutination tests on patients with pyrexia in whom typhoid, paratyphoid, influenza, pyogenic and tubercular infections had been excluded. They found in one case agglutination tests varied from no complete agglutination with a *melitensis* strain to 1:1,600 complete with one *abortus* strain and 1:100 with another. The organism isolated from the patient's blood was *Brucella abortus.* Previously, in 1932, Plastridge and McAlpine,[4] who employed agglutination absorption technique on 116 strains of *abortus,* indicated close agreement of antigenicity of strains, 85 per cent of which completely absorbed agglutinins from antisera of bovine and porcine types and from one melitensis antiserum. In their own words, "No serologic differences were observed between bovine and porcine types of *Brucella abortus,* regardless of origin." Feusier and Meyer,[5] as early as 1920, concluded, "Strains within the same group do not necessarily act in a uniform manner when absorbed from the same antiserum. This constitutes the basis for individual differentiation. In several *melitensis* strains, for example, the range of variability was so great that certain strains appeared to have little in common." Giordano,[6] Keefer,[7] Carpenter, Boak, and Chapman,[8] and Foshay[9] mention specifically that in routine testing variations in agglutination titers of patients were marked when separate strains were used to check. Agglutination absorption test and standard agglutination test apparently are not comparable, since from our results with *abortus, suis,* and *melitensis* strains we concluded that infecting Brucella strains must vary tremendously in antigenicity.

Huddleson[1] states that in routine testing an antigen containing one smooth strain of abortus is sufficient. To determine the smoothness of the strain, the method of choice is the phagocytosis of the strain with known negative and positive bloods as an index. Our strains were neither thermoagglutinable nor dye agglutinable and yet variation in phagocytic system was marked. We feel that variation in the phagocytic system was a strain difference, not necessarily associated with its smoothness or roughness.

CONCLUSIONS

1. A series of 34 patients diagnosed as having chronic undulant fever has been examined, employing agglutination and opsonocytophagic tests.

2. Marked variation in both agglutinin titers and opsonocytophagic indices has been found. This indicates the necessity for standardization of Brucella polyvalent antigens for agglutination tests.

3. Testing with five to ten smooth strains for cytophagic activity is necessary to obtain reliable results.

4. From our studies it would appear that the diagnosis of acute or chronic brucellosis is dependent upon clinical opinion with some positive immunologic test.

REFERENCES

1. Huddleson, I. Forest: Brucellosis in Man and Animals, New York, 1939, The Commonwealth Fund.
2. Foshay, Lee, and LeBlanc, T. J.: The Derivation of an Index Number for the Opsonocytophagic Test, J. Lab. & Clin. Med. **22**: 1297, 1937.
3. Campbell, William, and Greenfield, E. C.: The Incidence of Brucellosis in Cases of Pyrexia of Uncertain Origin in the Cape Province of the Union of South Africa, South African M. J. **11**: 192, 1937.
4. Plastridge, W. N., and McAlpine, J. G.: Agglutinin-Absorption Studies on Brucella, J. Infect. Dis. **50**: 555, 1932.
5. Feusier, M. L., and Meyer, K. F.: Principles in Serologic Grouping of Br. Abortus and Br. Melitensis: Correlation Between Absorption and Agglutination Tests, J. Infect. Dis. **27**: 185, 1920.
6. Giordano, Alfred S., and Sensenich, R. Lloyd: Brucella Abortus Infection in Man. A Clinical Analysis of 35 Cases, J. Lab. & Clin. Med. **15**: 421, 1930.
7. Keefer, Chester: Report of a Case of Malta Fever Originating in Baltimore, Md., Bull. Johns Hopkins Hosp. **35**: 6, 1924.
8. Carpenter, G. M., Boak, Ruth, and Chapman, O. D.: Significance of Brucella Abortus Agglutinins in Human Serum, J. Immunol. **17**: 65, 1929.
9. Foshay, Lee: The Diagnosis of Undulant Fever, Am. J. Clin. Path. **10**: 176, 1940.

RECOVERY OF RABIES VIRUS FROM THE BRAIN OF AN UNDIAGNOSED CASE*

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THE symptoms of rabies are usually so typical that when accompanied by a history of a dog bite, the case will appear to follow the classical description closely, and the diagnosis is rarely missed. When, however, a history of a bite is not previously established, and a case presents itself during a late stage of the disease, a correct diagnosis may then become exceedingly difficult. It is possible, therefore, that an occasional death which follows a symptomatology obscured by the lack of an incriminating history of contact with a rabid animal, may actually be the result of infection with rabies virus.

The belief has been expressed that this may occur much more frequently than may be supposed.[1-3] An excellent illustration of such an occurrence is described in the following report, which deals with the isolation and identification of rabies virus from a case that came to the Medical Examiner for autopsy because the symptomatology and cause of death were obscure.

In the afternoon of Feb. 15, 1940, S. G., white, 49 years old, residing on the lower East Side of New York, walked into the Columbus Hospital and asked to be admitted for observation. He complained of difficulty in swallowing, which he stated was "because of poison in

*From the Bureau of Laboratories, Department of Health, New York City.
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throat placed there by someone.'' After admission the patient became unmanageable, wildly delirious, and induced emesis which resulted in the vomiting of black fluid mixed with blood. The diagnosis was ''psychoneurosis'' and ''acute state of anxiety.'' He was about to be transferred to the psychopathic ward at Bellevue Hospital, when he died at 10:00 A.M. on February 16. The body was submitted to the Medical Examiner, and a post-mortem examination was made by Dr. Eugene Clark on February 17.

There was no evidence of injury, except for abrasions on the dorsum of the hands, on the middle finger of the right hand, and a healing wound, irregularly shaped and 1½ inches long, on the right index finger. The meninges were normal. There was a slight subarachnoid hemorrhage at the posterior pole of the cerebrum. No macroscopic lesions were found in the pons, medulla, or elsewhere in the brain. Aside from a general visceral congestion, no special pathology could be observed. Opinion as to the cause of death was, therefore, reserved, pending chemical and microscopic examination.

On the day of autopsy portions of the brain in glycerin and in 10 per cent formalin were submitted to us for investigation of a possible central nervous system virus infection.

For aid in the pathologic examination of the fixed tissues, we secured the assistance of Dr. Vera B. Dolgopol, of the Willard Parker Hospital. Her opinion was: ''Sections from medulla show a number of veins with several rows of lymphocytes within the walls. The perivascular space is free from exudate, but is markedly dilated. Some of the veins with cellular exudate are situated near the ventricular surface, while others are present on the lateral aspect of the medulla and near the olives. No glial nodules are observed. The ganglion cells show no remarkable degeneration. The section from the brain shows marked congestion of veins but no vascular infiltration. Microscopic diagnosis: bulbar myelitis.''

Because it was inconvenient to do animal inoculations at the time the glycerinated brain material was received, it was washed several times in saline solution to remove the glycerin, then frozen and stored in a dry-ice cabinet at −76° C. for forty-eight hours. The portions of brain were then thawed rapidly in the incubator at 37° C., titurated in a mortar to a fine pastelike consistency, and suspended in broth to a concentration of about 20 per cent. The suspension was centrifuged at about 2,000 r.p.m. for ten minutes, and the supernatant fluid was inoculated intracerebrally into animals. Each of six mice received 0.03 c.c., each of two guinea pigs 0.25 c.c., and each of two rabbits 0.5 c.c. Cultures in broth were made simultaneously to determine the bacterial content of the suspension. The results of these inoculations and all successive passages made thereafter are indicated in Fig. 1.

All material for subsequent inoculations consisted of a 10 per cent suspension of a pool of several mouse brains or, as in the one case indicated, of a single guinea pig brain. The volumes inoculated throughout were the same as those stated above.

The two inoculated guinea pigs were found dead the following morning. Smears and cultures from the brains of these animals showed heavy bacterial growth, with predominance of a gram-positive bacillus and fewer gram-positive cocci. These were also present in cultures of the original brain material.

Fortunately, the mice resisted these contaminants, and on the ninth day after inoculation two mice became inactive and showed a weakness of the hind legs. Several hours later they became moribund and were thereupon promptly killed. Their brains were removed with aseptic precautions, a portion of each was inoculated into culture media and the remainder was frozen at −76° C. As

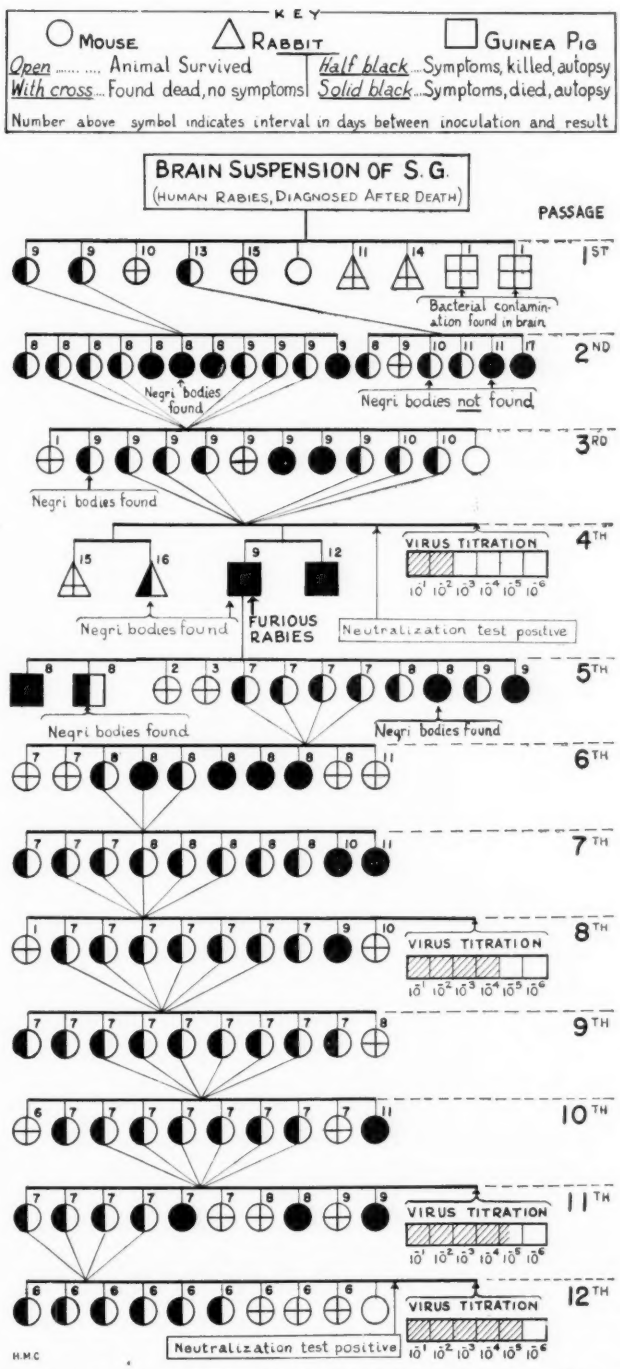

Fig. 1.—Animal passages and tests with virus isolated from a case of human rabies.

no growth was observed in the cultures, a 10 per cent suspension of these brains was prepared and inoculated into a group of ten mice. On the thirteenth day a third mouse of the originally injected group of six showed symptoms similar to those observed in the first two, and a suspension of its brain proving bacteriologically sterile was inoculated into a group of six mice.

The two rabbits were found dead in their cages on the eleventh and fourteenth day, respectively. Bacteriologic examination of the brains of both these animals yielded negative results, and they were stored at –76° C., pending the outcome of the mouse passages.

Beginning with the second passage, the mice began to show more definite symptoms, usually starting with paresis, rapidly developing paralysis, and terminating in prostration and death. Since serial passage of the brains of animals thus affected resulted in a clear-cut, reproducible clinical syndrome, we inferred that an infectious agent, probably a neurotropic virus, was involved.

Judging from the case history, incubation period, and clinical symptoms in mice, the viruses first to be considered logically appeared to be those of rabies, herpes, or encephalitis.

Accordingly, the brain of one mouse in the second passage was examined for Negri bodies by direct smear, and two brains were sectioned for histopathologic study. No Negri bodies were found on direct smear. The histologic sections showed mononuclear cuffing around small veins in the subcortical structures and the cortex. There was no diffuse meningitis, although occasionally groups of lymphocytes were present in limited areas of the meninges. A large number of intracellular and extracellular Negri bodies were seen in one of the sections of the hippocampus.

In subsequent passages, namely, the third, fourth, and fifth, Negri bodies were easily demonstrated by direct smear. These were seen also in the brains of a rabbit and a guinea pig* inoculated with third passage mouse brain and in the brain of another guinea pig receiving a further guinea pig brain passage. None of the brains obtained in the remainder of passages was examined for Negri bodies.

The finding of Negri bodies made it fairly obvious that rabies virus had been isolated. It was nevertheless deemed necessary to conduct neutralization tests in order to demonstrate the serologic specificity of this virus. The virus was, therefore, tested against an antirabic serum prepared by the injection of rabbits with a Department of Health strain of fixed rabies virus. The serum definitely neutralized the isolated virus in this test, but because of the low infectivity of the virus in its third passage, further passages were resorted to so that the potency of the virus might increase sufficiently and a repetition of the neutralization test could be made under more suitable conditions.

The chart of the virus passages shows quite clearly that a gradual rise in the infectivity titer took place, starting at 10^{-2} in the third passage to 10^{-5} in the eleventh passage. It will also be noted that correspondingly there was a decrease in the incubation period from nine days or more at the beginning to a uniform six-day incubation period in the last passage made.

*This animal exhibited symptoms characteristic of furious rabies. It became excitable, ran wildly about in its cage, frothed at the mouth, and repeatedly attacked and bit its cage mate. Finally, after repeated convulsive seizures, it became prostrate and died.

A virus preparation obtained from a pool of eleventh passage mouse brains was again tested against the antirabic serum. The results of the second test fully confirmed the findings of those in the first in a clear-cut and satisfactory manner.

As soon as sufficient evidence was obtained to be certain that we had uncovered a case of undiagnosed rabies, further investigation was made by the Bureau of Preventable Diseases, and the following facts were elicited:

From an interview with the son of S. G. it was learned that S. G., while at play with his dog on December 25, was bitten on the index finger of the right hand. He applied tincture of iodine and a bandage to the bleeding wound, but sought no medical advice. The son, P. G., was bitten on the left leg on the same day. He also applied tincture of iodine with no further treatment. The dog subsequently vanished and was not seen thereafter, but P. G. added the further information that the dog had been ill for two days prior to December 25 with vomiting and "fits," and had tried to bite people on the street. He also stated that the dog showed scars on its face as a result of having been bitten by another dog two weeks previously.

S. G. felt well and worked as a laborer until February 13 when he developed fever. On February 14 he could not drink or swallow, had dryness of the mouth, and feared the sight of water. A local physician examining him that day diagnosed his condition as that of grippe.

On February 15 a Hospital ambulance interne refused to remove him to the hospital, stating he was not sick enough for hospitalization. The same day S. G. went to Columbus Hospital where he was admitted. Death ensued the following day.

This information, furnished by the Bureau of Preventable Diseases, served to complete the history of a perfectly typical case of rabies which, baffling as it appeared in the beginning, might have remained undetermined and unrecorded were it not for the first clues obtained in the laboratory.

To the physician and public health officer this report may be of significance, since it indicates the need to be more watchful for cases of unsuspected rabies in areas where this disease is endemic.

SUMMARY

With the utilization of laboratory procedures, rabies virus was recovered from a person, the cause of whose death was obscure. The presence of virus in the brain of this undiagnosed case was established by animal passages, by the demonstration of Negri bodies in the brains of inoculated animals, and by immunologic methods.

Following the recovery of virus, further investigation revealed that the history of a dog bite and other data pertinent to a typical case of rabies also existed.

The need to be ever mindful that such cases may occur in communities where rabies is endemic is indicated by this report.

REFERENCES

1. Geiger, J. C., and Rusk, G. Y.: Human Rabies in California, With Discussion of Differential Microscopical Anatomical Diagnosis, California & West. Med. **49**: 197, 1938.
2. Millischer, P., Dopf, A., and Mazet, J. B.: Observation d'un cas de rage humaine d'origine indéterminée, Soc. de méd. mil. franç., Bull. mens. **31**: 496, 1937.
3. Webster, L. T.: Epidemiologic and Immunologic Experiments on Rabies, New England J. Med. **217**: 687, 1937.

SULFONAMIDE THERAPY OF STREPTOCOCCUS INFECTIONS BY THE INTRAVENOUS DRIP METHOD*

With a Note on the Toxicity of Neoprontosil by Intravenous Administration

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With the Technical Assistance of Anna M. Rule and Loraine Groskin

THE massive arsenotherapy of early syphilis by the intravenous drip injection of solutions of neoarsphenamine or mapharsen over a period of five days, proposed by Chargin, Leifer, and Hyman,[1, 2] has suggested that this method of administration of the soluble sulfonamide compounds may be useful in the treatment of streptococcal and other septicemias.

However, since therapeutically effective concentrations of free sulfonamide compounds in the blood may be maintained in the majority of persons by oral or parenteral administration of adequate doses at suitable intervals, the indications for their administration by the continuous intravenous drip method are not apparent, although worthy of investigation in relation to the chemotherapy of severe septicemia with special reference to the treatment of subacute bacterial endocarditis due to infection with *Streptococcus viridans*.

In this connection it may be stated that the massive arsenotherapy of acute testicular syphilis of rabbits by Kolmer and Rule,[3] consisting of the intravenous drip method of administration of neoarsphenamine and mapharsen once a day for five days in succession, did not prove any more effective than the daily intravenous injection of single doses by syringe for five days in succession, or single intravenous injections of the compounds, as the *total* minimal curative doses in relation to biologic cure were practically the same by all three methods of administration, being approximately 0.020 Gm. of neoarsphenamine or 0.005 Gm. of mapharsen per kilogram of weight.

EXPERIMENTAL

Rabbits were chosen for the experimental part of this investigation because of their susceptibility to infection with virulent beta hemolytic streptococci of group A by intradermal or intravenous inoculation[4] and the ease with which solutions of sulfanilamide and neoprontosil could be injected intravenously by the drip method at daily intervals.

*From the Research Institute of Cutaneous Medicine, Philadelphia.
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On the other hand, it was realized that rabbits are not as suitable as mice for chemotherapeutic investigations in experimental streptococcus infections with the sulfonamide compounds largely because sulfanilamide and other sulfonamide compounds are more rapidly acetylated into compounds of reduced or doubtful therapeutic properties.[5, 6] Thus Kolmer, Brown, and Rule[7] have observed that about 83 per cent of untreated rabbits inoculated intradermally and intravenously at the same time with virulent beta hemolytic streptococci developed suppurative arthritis, which progressed to a fatal outcome with positive blood and joint cultures. In a series treated with sulfanilamide by intravenous administration about 75 per cent developed suppurative arthritis with positive blood and joint cultures, all of which terminated fatally. Better results, however, were observed in the treatment of rabbits with neoprontosil inoculated only intradermally, which produces a severe local lesion without an associated septicemia.[8]

The strain of beta hemolytic streptococcus (group A) employed in this investigation was of such virulence that the intravenous injection of adult rabbits, weighing from 2,000 to 2,800 Gm., with 0.8 c.c. of 18-hour broth culture, regularly produced infections fatal within fourteen days with the development of suppurative arthritis with positive joint cultures in about 75 per cent of animals. Heart blood cultures were usually positive at daily intervals during the first four or five days following inoculation after which they were usually sterile.

TABLE I

RESULTS OF TREATMENT OF RABBITS WITH SULFANILAMIDE AND NEOPRONTOSIL ADMINISTERED BY THE INTRAVENOUS DRIP METHOD*

COMPOUNDS	NUMBER RABBITS†	DOSE PER KG. (GM.)	SURVIVALS: DAYS						NUMBER SURVIVALS
			2	4	6	8	10	14	
Sulfanilamide	3	0.020	3	3	3	2	2	0	0
Sulfanilamide	3	0.050	3	3	2	1	1	0	0
Sulfanilamide	3	0.100	3	3	3	2	2	0	0
Sulfanilamide	3	0.200	3	3	3	2	1	0	0
Neoprontosil	3	0.020	3	2	2	1	0	0	0
Neoprontosil	3	0.050	3	3	2	2	1	0	0
Neoprontosil	3	0.100	3	3	3	2	1	0	0
Neoprontosil	3	0.200	3	2	2	1	0	0	0
Untreated controls	8	-	8	8	8	6	4	0	0

*Administered once daily for five days in succession. Each dose of both compounds was given in 80 c.c. of 5 per cent solutions of glucose. Time required for injections varied from three hours and forty-seven minutes to four hours and two minutes. First dose of each compound was given twenty-four hours after inoculation.

†Weights 2,400 to 2,800 Gm. Inoculated intravenously with 0.8 c.c. of eighteen-hour broth culture of virulent beta hemolytic streptococcus.

In one experiment 32 rabbits were inoculated in this manner with 8 being kept as untreated controls. Of the remaining 24 animals 12 were given sulfanilamide and 12 neoprontosil intravenously by the drip method at daily intervals for five days in succession, the first dose of each compound being given twenty-four hours after inoculation, at which time positive heart blood cultures were observed. The daily doses of each compound varied from 0.020 to 0.200 Gm. per kilogram of weight, equivalent to 1.4 to 14.0 Gm. per 70 kg. of weight. Each dose was dissolved in 80 c.c. of a 5 per cent solution of glucose and given intravenously by the drip method, each treatment requiring about four hours. No difficulties were encountered in giving the injections by the gravity method

through the veins of the ears, the animals being confined in a special box in a comfortable position without the need of employing anesthesia. The results are summarized in Table I.

A second series of 32 rabbits was inoculated in the same manner, 8 being kept as untreated controls, and the remaining 24 treated with sulfanilamide and neoprontosil in the same dosage per kilogram of weight except that each compound was administered daily by intravenous injection by the syringe method at the rate of 5 c.c. per minute. Sulfanilamide was employed in an 0.8 per cent solution which required the injection of from 6 to about 48 c.c. in order to administer doses of 0.020 to 0.200 Gm. per kilogram of weight. In the case of neoprontosil a stock 5 per cent solution was employed so diluted with sterile water that each dose was administered in a volume of 15 c.c. The results are summarized in Table II.

TABLE II

RESULTS OF TREATMENT OF RABBITS WITH SULFANILAMIDE AND NEOPRONTOSIL ADMINISTERED BY THE SYRINGE METHOD*

COMPOUNDS	NUMBER RABBITS†	DOSE PER KG. (GM.)	SURVIVALS: DAYS						NUMBER SURVIVALS
			2	4	6	8	10	14	
Sulfanilamide	3	0.020	3	3	2	1	0	0	0
Sulfanilamide	3	0.050	3	3	3	2	0	0	0
Sulfanilamide	3	0.100	3	3	3	3	3	0	0
Sulfanilamide	3	0.200	3	3	3	2	1	0	0
Neoprontosil	3	0.020	3	3	3	2	0	0	0
Neoprontosil	3	0.050	3	3	3	2	0	0	0
Neoprontosil	3	0.100	3	3	3	2	1	0	0
Neoprontosil	3	0.200	3	3	3	3	2	0	0
Untreated controls	8	-	8	8	7	6	3	0	0

*Administered once daily for five days in succession. Each dose of sulfanilamide was dissolved in 6 to 48 c.c. of 5 per cent solutions of glucose. Each dose of neoprontosil was prepared of a stock 5 per cent solution diluted with sterile distilled water to a total volume of 15 c.c. First dose of each compound was given twenty-four hours after inoculation.

†Weights 2,000 to 2,400 Gm. Inoculated intravenously with 0.8 c.c. of eighteen-hour broth culture of virulent beta hemolytic streptococcus.

Determinations of free sulfanilamide in the blood were made about one hour after the completion of the second treatment by both the drip and syringe methods of administration, and about two hours after the fourth treatment by both methods. The average results are summarized in Table III. It will be noted that the concentrations after the syringe method of administration were frequently higher than after the continuous drip method of administration, probably because of greater elimination of both compounds during the four hours required for their administration by the latter method.

TOXICITY OF SULFANILAMIDE AND NEOPRONTOSIL BY INTRAVENOUS ADMINISTRATION

Toxicity of Sulfanilamide.—Sulfanilamide was well borne by both methods of administration. All of four normal rabbits given the compound in doses of 0.020, 0.050, 0.100, and 0.200 Gm. per kilogram, respectively, at daily intervals for five days in succession by the intravenous drip method in the same manner as employed in the therapeutic experiments, survived indefinitely, with no clinical evidences of toxicity. The same was true of four normal controls given the

compound in similar dosage daily for five days in succession by the syringe method of intravenous injection with no evidences of the "speed shock" observed by Hirschfeld, Hyman, and Wanger[9] in relation to the intravenous injection of the organic arsenical and other compounds, probably because the sulfanilamide was administered in an 0.8 per cent solution.

TABLE III

CONCENTRATIONS OF FREE SULFANILAMIDE IN THE BLOOD*

COMPOUNDS	DOSE PER KG. (GM.)	METHOD OF ADMINISTRATION	AVERAGE BLOOD CONCENTRATION AFTER	
			2ND DOSE	4TH DOSE
Sulfanilamide	0.020	Drip	1.1	3.1
Sulfanilamide	0.020	Syringe	2.2	1.2
Sulfanilamide	0.050	Drip	3.5	3.1
Sulfanilamide	0.050	Syringe	1.6	1.2
Sulfanilamide	0.100	Drip	4.0	3.7
Sulfanilamide	0.100	Syringe	1.5	1.6
Sulfanilamide	0.200	Drip	12.05	5.3
Sulfanilamide	0.200	Syringe	7.1	5.6
Neoprontosil	0.020	Drip	0.5	0
Neoprontosil	0.020	Syringe	1.5	0.8
Neoprontosil	0.050	Drip	0.5	0.5
Neoprontosil	0.050	Syringe	0.5	0.5
Neoprontosil	0.100	Drip	0	0.5
Neoprontosil	0.100	Syringe	0.5	0.8
Neoprontosil	0.200	Drip	0.8	Negative
Neoprontosil	0.200	Syringe	3.6	1.8

*Milligrams per 100 c.c. Determinations were made about one hour after the completion of injections by the drip method and about two hours after injections by the syringe method.

Toxicity of Neoprontosil.—Of particular interest, however, was the fact that neoprontosil was likewise well borne by intravenous injection. All of four normal rabbits given 0.020, 0.050, 0.100, and 0.200 Gm. per kilogram, re-respectively, at daily intervals for five days in succession by the intravenous drip method in the same manner as employed in the therapeutic tests, survived indefinitely with no clinical evidences of toxicity. The same was true of four additional animals given the compound in the same dosage daily for five days in succession by the syringe method of administration, each dose being diluted with sterile water to a total volume of 15 c.c.

However, since neoprontosil is not customarily administered to human beings by intravenous injection, we have thought it advisable to conduct additional toxicity tests by this route of parenteral administration.

For this purpose normal rabbits were given neoprontosil* intravenously in 5 per cent solution twice daily (10 A.M and 3 P.M.) for eight days in succession in doses of 0.1, 0.2, 0.5, and 1.0 Gm. per kilogram by syringe injection, two animals being used for each dose. The total daily dosage varied, therefore, from 0.2 to 2.0 Gm. per kilogram or from 1.6 to 16.0 Gm. per kilogram over the eight days of administration and equivalent to from 14.0 to 140.0 Gm. per day for a human adult weighing 70 kg. As shown in Table IV, all animals survived indefinitely except one which died after six doses of 1.0 Gm. each or a total of 6.0 Gm. per kilogram over three days of administration.

*We are indebted to the Department of Medical Research, Winthrop Chemical Company, for kindly supplying the compound.

All surviving animals were killed one week after the last dose and microscopic examinations were made of the liver, spleen, kidneys, and sternal bone marrow of each. No pathologic tissue changes were found in the sections of the spleens or bone marrow. The livers of two animals showed slight cloudy swelling with perilobular congestion, but no fatty degeneration or necrosis of the peripheral lobular cells. The kidneys of only one animal showed some dilatation of the convoluted or collecting tubules associated with tubular necrosis of minor degree. The histopathologic changes, therefore, were much less marked than observed by Rake, VanDyke, and Corwin[10] following the oral administration of sulfathiazole and sulfapyridine to mice, rats, and monkeys and also less than observed by Kolmer, Rule, and Groskin[11] following the oral administration of sulfathiazole and sulfathiazoline to rabbits.

TABLE IV

TOXICITY OF NEOPRONTOSIL FOR RABBITS BY INTRAVENOUS ADMINISTRATION

WEIGHT (GM.)	GM. PER KG. TWICE DAILY*	TOTAL PER KG. (GM.)	RESULTS	BLOOD CONCENTRATIONS (MG. PER 100 C.C.)†				
				1ST DOSE	3RD DOSE	5TH DOSE	9TH DOSE	15TH DOSE
2,000	0.1	1.6	Survived	0.16	0.12	0.16	0.16	0.14
1,950	0.1	1.6	Survived	Negative	Negative	0.08	0.08	0.04
1,850	0.2	3.2	Survived	0.16	0.16	0.24	0.24	0.14
1,900	0.2	3.2	Survived	Negative	0.12	0.16	0.16	0.14
1,750	0.5	8.0	Survived	0.28	0.32	0.36	0.40	0.32
1,800	0.5	8.0	Survived	0.28	0.28	0.36	0.38	0.32
1,700	1.0	6.0	Died 4th day	0.40	0.72	0.60	0	0
1,700	1.0	16.0	Survived	0.50	0.56	0.56	0.60	0.64

*At 10 A.M. and 3 P.M. for eight days in succession; 5 per cent solution of neoprontosil was employed.

†Determinations were made four hours after administration.

Total erythrocyte and leucocyte counts were made just before and four hours after the second, sixth, tenth, and sixteenth doses in each rabbit. Allowing for physiologic variations in different animals, the results have shown no effect upon the erythrocytes and leucocytes of those given 0.2 and 0.4 Gm. per kilogram per day. In those animals given 1.0 and 2.0 Gm. per kilogram per day a slight but definite decrease of the total erythrocytes per cubic millimeter of blood was noted, especially in the case of the animal that died after six doses of 1.0 Gm. each per kilogram, in which the total erythrocytes had dropped from 6,070,000 per cubic millimeter before the first dose to 4,200,000 four hours after the sixth dose. None of the animals, including that which died, showed a leucopenia; on the contrary, most of them showed a tendency to slight leucocytosis.

Blood examinations for the determination of free sulfanilamide were made four hours after the first, third, fifth, and fifteenth injections of the compounds in each rabbit. As shown in Table IV, the concentrations were quite low, evidently due in part to rapid acetylation, and usually much less than those observed one hour after administration, shown in Table III.

Agglutination tests in vitro, employing 1 c.c. of a 1 per cent suspension of washed human erythrocytes with amounts of 5 per cent solution of neoprontosil, varying from 0.1 to 1.0 c.c., and the addition of physiologic saline solution to a total volume of 2 c.c. showed some agglutination (+ to +++) upon microscopic examination of each mixture after incubation in a water bath for two hours at

37° C., but in spite of this evidence of the agglutination of washed erythrocytes in vitro no clinical evidences of intravascular agglutination or hemolysis were apparent in vivo in the case of rabbits, probably because of the protection afforded by the plasma proteins.

THERAPEUTIC RESULTS IN THE TREATMENT OF RABBITS

As shown in Tables I and II, both sulfanilamide and neoprontosil administered once daily for five days in succession by the intravenous drip and syringe methods of administration proved ineffective in the treatment of rabbits inoculated intravenously with virulent beta hemolytic streptococcus. All treated animals, including 16 untreated controls, died within a period of fourteen days. All developed suppurative arthritis with positive joint cultures at the time of death.

These results were not unexpected in the case of those animals given the compounds intravenously once a day by syringe injection in view of the unfavorable results previously observed following the oral administration of sulfanilamide to rabbits developing this type of streptococcus infection.[7] The main purpose of the investigation was to determine whether or not better results could be secured by the intravenous drip method of administration, but such has not been observed in these experiments. It is likely that failure of treatment by both methods of administration was largely due to the lack of maintenance of therapeutically effective concentrations of free sulfanilamide in the blood because of rapid acetylation of sulfonamide compounds by rabbits, along with the fact that only single doses were given once daily for five days in succession. It was hoped, however, that the intravenous drip method of administration, covering a period of about four hours for the injection of each dose, might prove superior, but such has not been observed.

THERAPEUTIC RESULTS IN THE TREATMENT OF HUMAN BEINGS

Both sulfanilamide and neoprontosil, however, may be safely administered to human beings by the intravenous drip method. The same is true of the soluble sodium salt of sulfapyridine. At least one of us (J. A. K.) has administered sulfanilamide by this method to five adult patients with severe surgical hemolytic streptococcus septicemia in whom oral administration was unsatisfactory because of nausea and vomiting. There were no apparent ill effects. Each treatment consisted in the intravenous injection of a mixture of 600 c.c. of 0.8 per cent solution of sulfanilamide in sterile saline solution diluted with 600 c.c. of 5 per cent solution of glucose at the rate of about 200 c.c. per hour, covering a period of about six hours. This administered 4.8 Gm. and the injections were repeated at intervals of six hours (two per twenty-four hours, totaling 9.6 Gm.) for five days in succession. The therapeutic effects were very prompt and all recovered.

Three additional cases of postabortal hemolytic streptococcus septicemia were treated in the same manner with neoprontosil with no apparent ill effects and with complete recoveries. Each treatment consisted of the intravenous drip administration of 100 c.c. of a 5 per cent solution of neoprontosil diluted with 1,100 c.c. of 5 per cent glucose solution given at the rate of about 200 c.c. per

hour over a period of about six hours (5 Gm.). The injections were repeated at intervals of six hours (two per twenty-four hours, totaling 10 Gm.) for five days in succession. It is true that neoprontosil has not been customarily administered by intravenous injection, since it is stated that this frequently produces nausea, vomiting, and immediate bowel movements,[12] but such were not observed following the intravenous drip administration of the compound in our three cases by the method employed.

Sodium sulfapyridine has been given to two adult patients with subacute bacterial endocarditis due to infection with *Streptococcus viridans* with two to four positive blood cultures, respectively, before the institution of treatment. Each treatment consisted of the intravenous drip administration of 4 Gm. of the compound dissolved in 1,200 c.c. of sterile saline solution at the rate of about 200 c.c. per hour over a period of about six hours. The injections were repeated at intervals of six hours (two per twenty-four hours, totaling 8 Gm.) for eight days in succession. There were no ill effects, although one patient developed a very mild hematuria on the sixth day of treatment which may have been due to the compound. At the completion of the course treatment was continued for twenty-four days by oral administration of sulfapyridine combined with the intravenous injection of typhoid-paratyphoid vaccine every three days for eight doses, for the production of fever. In spite of temporarily negative blood cultures, both patients ultimately died of the disease.

SUMMARY

1. The intravenous drip administration of sulfanilamide and neoprontosil in doses of 0.020 to 0.200 Gm. per kilogram once a day for five days in succession was well borne by rabbits with no evidences of toxicity.

2. The intravenous injection of neoprontosil to rabbits by syringe in doses of 0.1 to 1.0 Gm. per kilogram twice daily for eight days in succession was well borne with no clinical evidences of toxicity.

3. The intravenous drip administration of *sulfanilamide* in dose of 4.8 Gm. (600 c.c. of 0.8 per cent solution diluted with 600 c.c. of 5 per cent glucose solution) at the rate of 200 c.c. per hour for six hours, repeated every six hours (two injections per twenty-four hours) for five days in succession, was well tolerated by human beings with hemolytic streptococcus septicemia and gave excellent therapeutic results.

4. The intravenous drip administration of neoprontosil in dose of 5 Gm. (100 c.c. of 5 per cent solution diluted with 1,100 c.c. of 5 per cent glucose solution) at the rate of 200 c.c. per hour for six hours, repeated every six hours (two injections per twenty-four hours) for five days in succession, was well tolerated by human beings with hemolytic streptococcus septicemia and gave excellent therapeutic results.

5. The intravenous administration of *sodium sulfapyridine* in dose of 4 Gm. dissolved in 1,200 c.c. of physiologic saline solution at the rate of 200 c.c. per hour for six hours (two injections per twenty-four hours) for eight days in succession was well tolerated by two cases of subacute bacterial endocarditis due to infection with *Streptococcus viridans* but with unsatisfactory therapeutic results.

6. The intravenous drip and syringe methods of administration of sulfanilamide and neoprontosil in doses of 0.020 to 0.200 Gm. per kilogram of weight once a day for five days in succession proved ineffective in the treatment of rabbits inoculated intravenously with virulent beta hemolytic streptococcus and developing suppurative arthritis with septicemia. Failure was largely ascribed to rapid acetylation of the compounds resulting in therapeutically ineffective concentrations of free sulfanilamide in the blood.

REFERENCES

1. Chargin, L., Leifer, W., and Hyman, H. T.: Studies of Velocity and Response to Intravenous Injections: Application of Intravenous Drip Method of Chemotherapy as Illustrated by Massive Doses of Arsphenamine in Treatment of Early Syphilis, J. A. M. A. **104**: 878, 1935.
2. Hyman, H. T., Chargin, L., and Leifer, W.: Massive Dose Arsenotherapy of Syphilis by Intravenous Drip Method: Five-Year Observations, Am. J. M. Sc. **197**: 480, 1939.
3. Kolmer, J. A., and Rule, A. M.: Massive Arsenotherapy in Acute Syphilis of Rabbits by the Continuous Intravenous Drip Method, Arch. Dermat. & Syph. **44**: 1055, 1941.
4. Kolmer, J. A., and Rule, A. M.: Experimental Streptococcus Infections in Rabbits for Therapeutic Investigations, J. Lab. & Clin. Med. **22**: 1097, 1937.
5. Marshall, E. K., Jr., Emerson, J., Jr., and Cutting, W. C.: Para-Aminobenzene-sulfonamide: Its Absorption and Excretion, J. A. M. A. **108**: 953, 1937.
6. Long, P. H., and Bliss, E. A.: The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds, New York, 1939, The Macmillan Co., page 71.
7. Kolmer, J. A., Brown, H., and Rule, A. M.: Toxicity, Therapeutic Activity and Mode of Action of Sulfanilamide in Experimental Streptococcus Infections of Rabbits, J. Lab. & Clin. Med. **24**: 164, 1938.
8. Kolmer, J. A., Brown, H., Raiziss, G. W., Rule, A. M., and Clemence, L. W.: The Chemotherapy of Experimental Streptococcus Infections of Rabbits With Special Reference to Pyridine Compounds and Prontosil Soluble, J. Pharmacol. & Exper. Therap. **61**: 253, 1937.
9. Hirschfeld, S., Hyman, H. T., and Wanger, J. J.: Influence of Velocity on Response to Intravenous Injections, Arch. Int. Med. **47**: 259, 1931.
10. Rake, G., VanDyke, H. B., and Corwin, W. C.: Pathologic Changes Following Prolonged Administration of Sulfathiazole and Sulfapyridine, Am. J. M. Sc. **200**: 353, 1940.
11. Kolmer, J. A., Rule, A. M., and Groskin, L.: The Pathologic Tissue Changes Produced by Sulfathiazole and Sulfathiazoline in Rabbits, J. Lab. & Clin. Med. **27**: 1043, 1942.
12. Long, P. H., and Bliss, E. A.: Para-Amino-Benzene-Sulfonamide and Its Derivatives, J. A. M. A. **108**: 32, 1937.

THE CHEMOTHERAPEUTIC STUDIES OF 2-SULFANILYL-3-5 DIHYDROTHIAZOLE (SULFATHIAZOLINE)*

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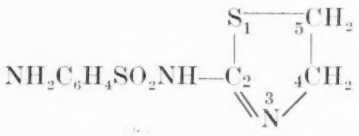
SULFATHIAZOLINE,[1] chemically 2-(p-aminobenzenesulfonyl)aminothiazoline,



differs from sulfathiazole in that the double bond in the latter, between carbon atoms 4 and 5, is saturated by the addition of an hydrogen atom to each of these carbon atoms. It is a colorless, crystalline compound, sparingly soluble in water, soluble in dilute mineral acids and alkalies. It has a melting point of 207 to 209° C.

TOXICITY

Mice tolerate more than 20 Gm. per kg. of body weight of sulfathiazoline given orally (Table I).

TABLE I

TOXICITY OF SULFATHIAZOLINE AND SULFATHIAZOLE ADMINISTERED TO MICE PER OS

Drugs Given in a 10 Per Cent Gum Acacia Suspension

DRUG	DOSE PER KG. (GM.)	NO. OF ANIMALS	SURVIVALS	
			NO.	PER CENT
Sulfathiazoline	10.0	20	20	100
	15.0	25	25	100
	20.0	50	49	98
Sulfathiazole	10.0	20	16	80
	15.0	24	17	71
	20.0	20	15	75

TABLE II

TOXICITY OF SULFATHIAZOLINE AND SULFATHIAZOLE ADMINISTERED TO RABBITS PER OS

Drugs Given in Water Suspension

DRUG	DOSE PER KG. (GM.)	NO. OF ANIMALS	SURVIVALS	
			NO.	PER CENT
Sulfathiazoline	3.0	12	12	100
	4.0	22	19	86
Sulfathiazole	4.0	9	5	66

According to Table II, sulfathiazoline was tolerated by 86 per cent of rabbits which received 4.0 Gm. per kilogram. Sulfathiazole, on the other hand, was tolerated by 66 per cent of rabbits which received 4.0 Gm. per kilogram.

*From the Dermatological Research Laboratories, Philadelphia, Division of Abbott Laboratories, North Chicago.

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Five dogs were given sulfathiazoline by mouth in a dose of 1.0 Gm. each daily for fifty days, and no diarrhea or vomiting was observed in any case. The dogs survived the fifty-day observation period and did not lose any weight (Table III).

TABLE III

TOXICITY OF SULFATHIAZOLINE IN DOGS GIVEN ORALLY

NO. OF DOG	WEIGHT IN LB. WHEN TEST WAS STARTED	WEIGHT IN LB. WHEN TEST WAS COMPLETED	DAILY DOSE (GM.)*	TOTAL GIVEN (GM.)
1	22¼	24	1.0	50†
2	21	22½	1.0	50
3	22½	26	1.0	50
4	21	21½	1.0	50
5	20¼	21½	1.0	50

*Daily dose of 1.0 Gm. for 50 consecutive days.
†No diarrhea or vomiting was observed in any of the animals.

Kolmer[1] found that sulfathiazoline, in doses of 0.05, 0.1, and 0.2 Gm. per kilogram twice daily for ten days in succession (20 doses) by oral administration, was well tolerated by rabbits.

No macroscopic or microscopic injury of the meninges, brain, or spinal cord of any of the animals, including those receiving the largest amount of the compound, was found.

No gross or microscopic injury of the kidneys, liver, or spleen in a dose of 0.05 Gm. per kilogram twice daily for 20 doses was observed.

There was evidence of slight injury of the kidneys, liver, and spleen in the case of some animals given a dose of 0.1 Gm. per kilogram orally twice daily for 20 doses.

We made comparative studies of sulfathiazoline and sulfathiazole in reference to blood concentrations. The perusal of figures in test A shows a figure of 2.59 to 1.11 mg. per cent for sulfathiazoline. For sulfathiazole we found the blood concentration ranging from 3.2 to 1.19 mg. per cent. When a higher dose, namely, 0.5 Gm. per kilogram was given, the blood levels, two hours after injection, were considerably higher (Table IV).

Therapeutic Effect in Pneumococcus Types II and III.—In these experiments mice were infected intraperitoneally with 10 to 200 M.L.D. of types II or III pneumococcus, of which the average minimum lethal dose was 0.5 c.c. of 1:1,000,000 to 1:10,000,000 dilution of broth culture.

The drugs were given by mouth in a dose of 10 mg. three times daily at 9 A.M., 5 P.M., and 12 P.M. for five days; at 9 A.M. and 5 P.M. on the sixth day; and at 9 A.M. on the seventh day, a maximum of 18 treatments. From Table V, which presents a summary of the results obtained in several experiments, one should note that at the end of four days the percentage of survivals was higher for sulfathiazoline, 78 per cent against 73 per cent for sulfathiazole. At the end of the seventh day, however, the percentage of survivals is higher for sulfathiazole than for sulfathiazoline. The former shows 19 per cent survivals, and the latter, 14 per cent. At the end of twenty-eight days, 5 per cent of sulfathiazole-treated mice survived against 2 per cent for sulfathiazoline. In type III pneumococcic infections the percentage of survivals is highest for sulfa-

TABLE IV

BLOOD LEVELS IN MICE

TEST	SULFATHIAZOLINE				SULFATHIAZOLE			
	MOUSE NO.	INTERVAL IN HOURS AT WHICH ANIMALS WERE KILLED	BLOOD LEVELS CALCULATED IN MG. %		MOUSE NO.	INTERVAL IN HOURS AT WHICH ANIMALS WERE KILLED	BLOOD LEVELS CALCULATED IN MG. %	
			FREE	CONJ.			FREE	CONJ.
A	1	2*	2.49	None	11	2*	3.20	None
	2	2	2.59	None	12	2	2.91	None
	3	4	1.56	0.36	13	4	1.68	None
	4	4	1.87	0.26	14	4	1.24	None
	5	6	1.40	None	15	6	1.42	None
	6	6	1.18	None	16	6	1.30	None
	7	8	1.30	None	17	8	1.60	None
	8	8	1.11	None	18	8	1.19	None
	9	24	1.19	None	19	24	1.67	None
	10	24	1.24	None	20	24	1.36	None
B	1	2 (After 1st dose)†	5.71	1.14	13	2 (After 1st dose)†	15.88	3.84
	2	2 (After 1st dose)	7.14	2.28	14	2 (After 1st dose)	7.66	1.16
	3	4 (After 1st dose)	2.43	1.21	15	4 (After 1st dose)	5.29	0.99
	4	4 (After 1st dose)	2.43	None	16	4 (After 1st dose)	4.34	1.58
	5	6 (After 1st dose)	2.86	None	17	6 (After 1st dose)	6.71	None
	6	6 (After 1st dose)	2.14	0.30	18	6 (After 1st dose)	6.57	0.76
	7	15 (After 2nd dose)	1.81	1.05	19	15 (After 2nd dose)	0.74	0.30
	8	15 (After 2nd dose)	0.86	0.28	20	15 (After 2nd dose)	1.03	0.29
	9	2 (After 3rd dose)	3.14	1.29	21	2 (After 3rd dose)	11.61	4.68
	10	2 (After 3rd dose)	4.57	0.86	22	2 (After 3rd dose)	8.82	5.88
	11	4 (After 3rd dose)	3.00	3.08	23	4 (After 3rd dose)	3.80	0.60
	12	4 (After 3rd dose)	2.07	1.14	24	4 (After 3rd dose)	7.33	1.15
C	1	2‡	5.35	None	13	2‡	12.86	None
	2	2	4.40	None	14	2	9.40	None
	3	4	1.33	None	15	4	3.27	None
	4	4	1.92	None	16	4	6.43	None
	5	6	0.93	0.31	17	6	3.63	None
	6	6	0.88	0.25	18	6	2.20	None
	7	8	0.52	0.25	19	8	0.45	0.25
	8	8	0.30	0.25	20	8	0.72	0.31
	9	12	0.73	None	21	12	0.31	0.28
	10	12	0.30	None	22	12	0.33	0.21
	11	24	0.77	0.73	23	24	0.78	0.65
	12	24	0.50	0.75	24	24	0.48	1.08

*Each mouse received only one dose of 0.002 Gm., which is approximately 0.1 Gm. per kilogram of body weight, in 10 per cent gum acacia solution.

†Each dose administered was of 0.010 Gm. per os in a 10 per cent gum acacia solution.
1st dose given at 10 A.M.
2nd dose given at 4 P.M.
3rd dose given at 10 A.M. next day.

‡Each mouse received one dose of 0.010 Gm. per os in a 10 per cent gum acacia solution.

TABLE V

THERAPEUTIC EFFECT IN TYPES II AND III PNEUMOCOCCIC INFECTION IN MICE

TYPE OF PNEUMOCOCCUS	DRUG	NO. MICE USED	PERCENTAGE OF SURVIVALS IN DAYS									
			1	2	3	4	5	6	7	14	21	28
II	Sulfathiazoline	170	100	100	90	78	48	24	14	2	2	2
	Sulfathiazole	105	100	95	85	73	54	33	19	6	5	5
	Controls	101	15	0								
III	Sulfathiazoline	31	100	86	65	35	26	13	3	3	3	3
	Sulfathiazole	33	94	82	58	27	18	15	6	3	3	3
	Controls	25	48	48	8	0						

thiazoline at the end of five days; i.e., 26 per cent for sulfathiazoline and 18 per cent for sulfathiazole. At the end of the twenty-eighth day the percentage of survivals is equal for both drugs.

THERAPEUTIC EFFECT IN STAPHYLOCOCCUS AUREUS INFECTIONS IN MICE

Mice were infected intravenously (saphenous vein) with 0.2 c.c. of a dilute saline suspension of F.D.A. strain No. 209 *Staphylococcus aureus*. Prior to the experiment the strain was passed through mice four times, the abscessed kidneys of the infected animals being removed at the time of death and recultured on plain agar. This increased the virulence of the strain so that 100 per cent of a series of at least 25 mice were killed within one to five days as a result of infection.

The mice were treated one and one-half, seven, twenty-four, and thirty-two hours after infection, then once daily for fourteen days, a total of 18 treatments per os. From Table VI one can see that the percentage of survivals for sulfathiazoline at the end of four days was 100; for sulfathiazole, 84. At the end of fourteen days the percentage of survivals for sulfathiazoline is higher than that for sulfathiazole—48 per cent as compared with 28 per cent. At the end of twenty-eight days 4 per cent of sulfathiazoline-treated mice survived as compared with 8 per cent of sulfathiazole.

TABLE VI

THERAPEUTIC EFFECT IN STAPHYLOCOCCUS AUREUS INFECTIONS IN MICE

DRUG	NO. OF MICE USED	PERCENTAGE OF SURVIVALS, DAYS									
		1	2	3	4	5	6	7	14	21	28
Sulfathiazoline	25	100	100	100	100	96	88	80	48	4	4
Sulfathiazole	25	100	88	88	84	80	76	72	28	16	8
Controls	25	100	96	80	16	0					

These results represent a summary of 5 individual experiments.

CONCLUSIONS

1. When tested on mice, rabbits, and dogs, sulfathiazoline proved to be lower in toxicity than sulfathiazole.

2. The therapeutic effect of sulfathiazoline in mice with type II and III pneumococcic infection was about the same as that observed with sulfathiazole.

3. The percentage of survivals of mice infected with *Staphylococcus aureus* and treated with sulfathiazoline was found to be higher in the first fourteen days of observation, while in periods of twenty-one and twenty-eight days sulfathiazole showed more survivals.

REFERENCES

1. The product discussed in this study was described in a paper presented before the Medicinal Division of the American Chemical Society at St. Louis, Mo., April, 1941, by the senior author (G. W. R.) and published in the J. Am. Chem. Soc. **63**: 2739, 1941, and **63**: 3124, 1941.

2. Kolmer, J. A., Rule, A. M., and Groskin, L.: The Pathologic Tissue Changes Produced by Sulfathiazole and Sulfathiazoline in Rabbits, J. LAB. & CLIN. MED. **27**: 1043, 1942.

THE SURFACE AREA OF THE HUMAN ERYTHROCYTE*

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IN THE study of the anemias it has been customary to consider the amount of hemoglobin, the number and size (volume and diameter) of the red blood cells, and the relationship between them. All this is based upon the assumption that the hemoglobin is evenly and diffusely distributed throughout the cell mass. However, evidence has been brought forth to show that the molecules of hemoglobin are deposited on the surface of the cell rather than within the cell body.[10] If this were the case, one might indeed imagine that this would increase the efficiency of hemoglobin as an oxygen carrier, its chief function. Emmons,[2] after determining and comparing the red blood cell count, volume, thickness, area, and the hemoglobin content of several species of animals, showed that, although these values varied considerably in different species, the total surface area of the red cells per unit volume of blood was remarkably constant in all of them. This fact also would favor the concept that hemoglobin is limited to the surface of the cell. The surface area then would seem to be an important factor in evaluating oxygen-carrying efficiency of the red blood cells in a case of anemia.

The earliest attempt to calculate the surface area of the red cell was by Burker,[2] who used the formula $A = \pi \frac{D^2}{2}$ (D representing the diameter), neglecting the thickness of the cell and assuming the cell to be flat. Values thus obtained were, on the face of it, untrue, since they were less than that of a sphere of the same volume—the form that displays the smallest area per volume.

If we assume the erythrocyte to be a short cylinder or flat disk, the area can be computed mathematically by a simple formula. Knowing the diameter, we can determine the top surface area by the formula πr^2 (r representing radius). The area around the cell is equal to the circumference ($2\pi r$) multiplied by the thickness. Since the volume equals the top area multiplied by the thickness, the latter can be computed by dividing the volume by the top area, or $T = \frac{V}{\pi r^2}$ (T representing thickness and V representing volume). The total area is twice the area of the top surface plus that around the cell. The formula then would be $A = 2\pi r^2 + 2\pi rT$ or $A = 2\pi r\ (r + T)$.

The red cell, however, has a characteristic curved configuration and so would exhibit a larger area. By the use of Guldin's theorems of Integral Calculus, Emmons[2] computed the surface area for several species of animals. The neces-

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sary values had to be obtained by actually constructing models of the red cells and taking direct measurements. Such a method is obviously a very laborious and impractical one. If the above-mentioned formula would give close enough results, we would have a very simple and practicable method.

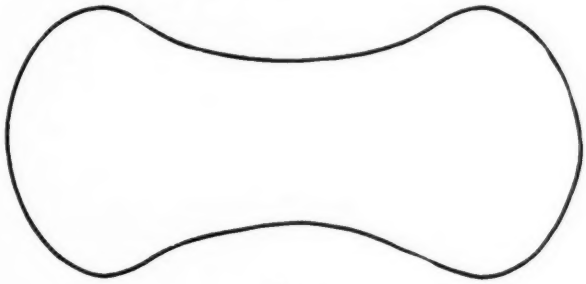

Fig. 1.

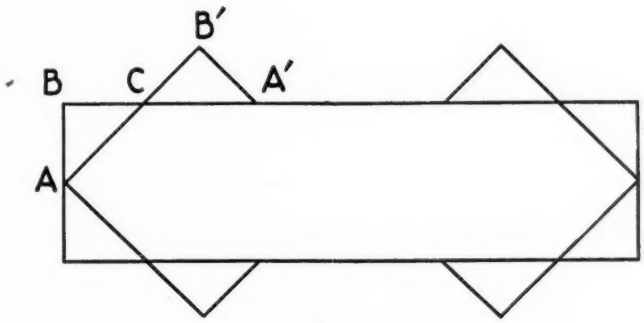

Fig. 2.

The object of this work is to develop a simple method of determining and establishing the normal value for the surface area of the human erythrocyte. This can then form a basis from which many of the hematologic relationships and implications can be investigated. We studied a series of 50 normal young adults (doctors, nurses, and hospital attendants), 27 males and 23 females.* All the determinations were done on venous blood. The anticoagulant used was prepared according to the method of Wintrobe as follows: 0.6 Gm. of ammonium oxalate and 0.4 Gm. of potassium oxalate was dissolved in 100 c.c. of water. One cubic centimeter of this solution was placed in each test tube, and the tube was then dried in the oven. This amount of anticoagulant was used for 10 c.c. of blood. The hemoglobin was determined by the Haden-Hausser method (100 per cent = 15.8 Gm. per 100 c.c.). The hematocrit was determined by the Sanford-Magath method. Cell measurements were made with the filar micrometer measuring to within 0.5 microns. Thickness was computed by dividing the mean corpuscular volume by πr^2, r being the mean radius of the cell. The formula used to compute the surface area was $A = 2\pi r(r + T)$, T being the thickness.

Table I represents our figures and results. The surface area of the entire series of cases ranged from 129.09 to 142.56 square microns, the average being

*Nine of the female subjects were nurses of the University Hospital, Ann Arbor, Mich. In these cases the hemoglobin was determined by the Sahli method and the hematocrit by the Wintrobe method.

TABLE I

HEMATOLOGIC VALUES IN THIS STUDY

NO.	NAME	R.B.C. COUNT (MILLIONS)	HB. (GM.)	HEMATOCRIT (C.C.)	MEAN CORPUSCULAR VOLUME (CUBIC MICRONS)	DIAMETER (MICRONS)	THICKNESS (MICRONS)	SURFACE AREA (SQUARE MICRONS)
1	L. K.	4.53	12.02	36.5	80.6	7.46	1.84	130.45
2	C. M.	5.03	14.11	44.0	87.5	7.14	2.18	129.09
3	E. L.	4.48	13.85	43.5	97.1	7.64	2.12	142.56
4	C. G.	4.98	13.06	42.5	85.3	7.59	1.88	135.28
5	M. U.	4.72	13.85	42.5	90.0	7.73	1.92	140.10
6	E. A.	4.66	13.77	40.0	85.8	7.66	1.86	137.00
7	M. F.	4.81	12.80	41.0	85.2	7.62	1.87	135.86
8	G. K.	4.43	12.93	40.0	90.3	7.84	1.87	142.50
9	M. F.	4.45	12.28	39.5	88.8	7.13	2.23	129.35
10	H. L.	4.64	13.00	43.0	92.7	7.38	2.17	133.48
11	M. H.	4.32	12.00	41.0	94.9	7.43	2.24	138.84
12	A. S.	4.67	13.00	42.0	89.9	7.39	2.10	134.44
13	E. K.	4.63	13.00	42.0	90.7	7.37	2.12	134.62
14	K. M.	4.46	12.50	41.0	91.9	7.23	2.24	130.36
15	M. H.	4.51	13.00	43.0	95.3	7.35	2.25	136.70
16	M. R.	4.58	12.50	41.0	89.5	7.35	2.10	133.58
17	J. M.	4.52	12.50	42.0	92.9	7.26	2.25	133.91
18	A. S.	4.67	13.00	44.0	94.2	7.44	2.17	139.59
19	L. B.	4.32	13.00	42.0	97.2	7.54	2.20	140.18
20	G. O.	4.63	13.00	42.0	90.7	7.39	2.11	139.00
21	D. C.	4.45	12.00	39.0	87.6	7.19	2.16	129.85
22	B. B.	4.80	13.50	44.0	91.7	7.58	2.02	138.89
23	M. R.	4.84	13.00	43.0	88.8	7.13	2.20	129.85
24	H. C.	5.13	14.50	47.0	91.6	7.16	2.27	131.51
25	K. C.	4.74	13.50	44.0	92.8	7.42	2.14	136.51
26	F. W.	4.96	14.00	47.0	94.8	7.60	2.10	140.77
27	A. A.	5.07	14.00	48.0	94.7	7.20	2.32	133.84
28	L. T.	4.30	12.50	40.0	93.0	7.55	2.10	139.59
29	A. K.	4.64	13.00	43.0	92.7	7.26	2.24	133.84
30	E. A.	4.88	14.50	46.0	94.2	7.26	2.28	134.75
31	N. W.	4.68	13.50	45.0	96.1	7.26	2.32	135.81
32	B. K.	4.87	13.50	45.0	92.4	7.30	2.20	134.68
33	S. M.	4.91	14.00	44.0	89.6	7.20	2.20	131.14
34	J. P.	5.21	15.00	50.0	95.9	7.42	2.22	138.29
35	I. P.	5.12	14.50	50.0	97.7	7.58	2.16	141.80
36	C. C.	5.27	15.50	48.0	91.1	7.60	2.01	138.65
37	E. S.	5.31	16.00	47.0	88.7	7.28	2.13	131.90
38	S. J.	4.89	13.50	45.0	92.0	7.28	2.20	135.50
39	H. B.	5.12	15.50	47.0	91.8	7.25	2.22	133.38
40	M. B.	5.10	14.00	48.0	94.1	7.27	2.26	134.89
41	W. B.	5.04	15.00	48.0	95.2	7.35	2.24	136.63
42	E. M.	4.95	14.00	46.0	92.9	7.20	2.28	132.93
43	D. L.	5.28	16.00	50.0	94.7	7.19	2.33	134.06
44	E. T.	5.06	15.00	49.0	96.8	7.41	2.25	138.62
45	H. S.	5.02	14.50	47.0	93.6	7.37	2.20	135.56
46	D. L.	4.93	14.00	44.0	89.1	7.28	2.14	132.13
47	F. B.	4.61	12.50	43.0	93.2	7.41	2.16	136.50
48	G. F.	4.93	14.50	46.0	93.3	7.37	2.21	135.09
49	G. L.	4.85	14.50	46.0	94.8	7.62	2.09	140.45
50	R. M.	4.72	13.00	43.0	95.2	7.20	2.34	134.29

135.44, with a standard deviation of 3.517. In 80 per cent of our cases the values ranged from 130 to 140 square microns. Table II represents the distribution of the cases for the various values obtained in our series. The value obtained by Emmons,[2] using this very exact but very laborious method described above, was 144.8 square microns.

We recognize the fallacies of our method. It is based on the assumption that the erythrocyte is a flat disk, while it is known that it has a curved configuration. But perhaps the fallacy is not quite as great as it may appear to be. Fig. 1 represents a cross section of the normal configuration of the red blood cell. Let us, for the sake of discussion, exaggerate the curves to the point of making straight lines to assume the appearance of Fig. 2. The rectangular figure superimposed is the flat disk used in our computations. The area A′B′C, omitted in our calculations, would be compensated for by the area A B C.

TABLE II

DISTRIBUTION OF CASES ACCORDING TO COMPUTED SURFACE AREA OF ERYTHROCYTES

SURFACE AREA IN SQUARE MICRONS	NUMBER OF CASES
129	4
130	2
131	3
132	2
133	7
134	7
135	6
136	4
137	1
138	6
139	2
140	4
141	1
142	2

We feel deeply indebted to Dr. Raphael Isaacs, formerly associate professor of medicine, University of Michigan, School of Medicine, Ann Arbor, Mich., for his guidance and valuable suggestions in carrying out this work.

To summarize, a practicable technique for determining the mean surface area of the human erythrocyte with the normal value and range is presented. By this method the average total surface area of the normal human erythrocyte is 135.44 ± 3.517 square microns. Eighty per cent of the cases ranged between 130 and 140 square microns. These results compare favorably with those obtained by more exact and much more difficult methods. This procedure opens possibilities for further investigations into the interrelationships of the surface area with other hematologic values and into the question of the distribution of hemoglobin in the red blood cell.

REFERENCES

1. Emmons, W. F.: Clinical Eriometer, Quart. J. Med. **21**: 83, 1927.
2. Emmons, W. F.: The Interrelation of Number, Volume, Diameter, and Area of Mammalian Erythrocytes, J. Physiol. **64**: 215, 1927.
3. Wintrobe, Maxwell, M.: The Erythrocyte in Man, Medicine **9**: 195, 1930.
4. Price, Jones, Vaughan, Janet M., and Goddard, Helen M.: Hematological Standards of Healthy Persons, J. Path. & Bact. **40**: 503, 1935.
5. Haden, R. L.: Volume-Thickness Index, J. LAB. & CLIN. MED. **20**: 567, 1935.
6. Ponder, Eric, and Millar, W. G.: Alterations in the Form of Mammalian Erythrocytes in Hypotonic Plasma, Quart. J. Exper. Physiol. **15**: 1, 1925.
7. Ponder, Eric, and Millar, W. G.: The Measurement of Diameters of Erythrocytes. I. The Mean Diameter of the Red Cells in Man, Quart. J. Exper. Physiol. **15**: 67, 1924.
8. Ponder, Eric, and Millar, W. G.: The Measurement of the Diameters of Erythrocytes. II. The Effect of Drying on the Diameter of the Red Cells in Man, Quart. J. Exper. Physiol. **14**: 319, 1924.
9. Ponder, Eric: The Erythrocyte and the Action of Simple Haemolysins, Edinburgh, 1924, Oliver & Boyd.
10. Garter, E., and Grendel, F.: On Bimolecular Layers of Lipoids on the Chromocytes of the Blood, J. Exper. Med. **41**: 439, 1925.

SARCOIDOSIS

A Consideration of the Clinical and Histologic Criteria Differentiating Sarcoidosis From Tuberculosis

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INTRODUCTION

JONATHAN HUTCHINSON[1] in 1875 was the first to call attention to a peculiar malady of the skin in a woman named Mortimer which was characterized by granulomatous infiltration and which he referred to as Mortimer's malady. Besnier,[2] fourteen years later, reported similar and more detailed clinical observations of this disease, but it was not until 1899 that Caesar Boeck[3] recorded the pathologic histology of these granulomatous lesions and described an associated lymphadenopathy. Many subsequent investigators frequently mentioned that the cutaneous lesions were accompanied by widespread adenopathy, dactylitis, and roentgenographic changes in the fingers and toes, yet Kuznitzky and Bittorf[4] in 1915 were the first to demonstrate conclusively diffuse sarcoid lesions and to establish definitely Boeck's sarcoidosis as a generalized pathologic process. Although their observations have been corroborated many times, the disseminated character of the disease is still far too often not appreciated.

Practically every organ in the body may be involved, but lesions occur most frequently in the skin, lymph nodes, bones of the hands and feet, and in the lungs. The skin eruption, as described by Boeck, is usually widespread, symmetrical, nodular, pigmented, and infiltrative but never ulcerative. It affects usually the face, ears, nose, and extensor surfaces of the extremities. The adenopathy may be generalized or limited, superficial or deep seated; it often occurs in persons who never develop lesions in the skin. When the enlargement of the lymph nodes is confined to a single group, the condition is usually diagnosed as tuberculosis and may even be considered Hodgkin's disease or lymphosarcoma. Frequently the earliest signs and symptoms are referable to the lungs where roentgenologically there may be observed a marked bilateral and symmetrical enlargement of the hilar and peribronchial lymph nodes, a soft infiltration resembling caseous pneumonic tuberculosis, and often a peculiar basilar infiltration suggesting miliary tuberculosis. In spite of such marked roentgenographic involvement, the clinical manifestations of pulmonary disease are exceedingly few, since only a mild febrile reaction accompanies transitory chest pains and a slight nonproductive cough. Hemoptysis does occur, but it is rare.

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The alterations in the bones of the hands and feet have been described adequately roentgenologically by Kienbock[5] and Jüngling[6] as punched out or reticulated areas of rarefaction in the medulla of the phalanges, with no involvement of the periosteum and joints.

In extensive studies of the blood Harrell[7] found definite increases in the content of calcium, protein, and phosphatase, but normal values for nonprotein nitrogen and phosphorus. In his series of cases Bence-Jones protein was usually present. In addition there were an increased sedimentation rate of the erythrocytes, a low or normal white blood cell count with neutropenia, eosinophilia, and relative monocytosis.

The disease occurs usually in early adult life and is generally benign, but it has been the cause of death in a few instances.[8, 9] In Longcope and Pierson's[10] group and in Harrell's series Negroes predominated. Although for years it has been felt that the disease was related to tuberculosis, no investigator has been able to isolate the tubercle bacillus from the lesion or through animal inoculation. At present it is almost universally agreed that the etiology remains obscure. That the disease does occur without any abnormalities of the skin has been demonstrated by many observers. It was absent in three of Harrell's eleven cases, while 50 per cent of those published by Longcope and Pierson failed to show cutaneous lesions. We are presenting three patients with Boeck's sarcoidosis, in none of whom at any time did skin manifestations appear. In two of our cases the lymph nodes in localized regions only were clinically affected, while in the third case there was widespread involvement of the lymph nodes, and in addition lesions of the liver and lungs. The failure of clinicians to consider Boeck's sarcoidosis in the differential diagnosis of the lymphadenopathies and also the failure of roentgenologists to include this disease in the differential diagnosis of pulmonary changes resembling tuberculosis, but which cannot be proved to be such by any of the many laboratory procedures now available for this purpose, are emphasized. Moreover, these cases show how easily the histologic picture may be misinterpreted by the pathologist who is not familiar with it.

CASE HISTORIES

CASE 1.—K. G., a 38-year-old white woman, had at the age of 12 years a lump in her neck which first appeared at the angle of the jaw on the right side. It reached the diameter of 1 cm.; it was painless and unassociated with any systemic manifestations, such as fever, loss of weight, etc. This lump persisted apparently unchanged for twenty years, until at the age of 32 it began to enlarge without any detectable lymphadenopathy elsewhere. At that time there were no accompanying signs of systemic disease, yet a tonsillectomy was deemed advisable and was performed six months later, on March 28, 1935. Because no appreciable effect was noted upon the enlarging mass in the neck following the operation, the mass was extricated on April 29, 1941. At operation a firm, encapsulated oval mass, 3 by 2 cm., was found lying beneath the superficial fascia of the neck, near the right carotid artery at the angle of the jaw. No alterations in the near-by lymph nodes were found, and nodes were not palpable elsewhere.

Upon gross section the incised mass appeared to be composed of lymphoid tissue surrounded by a well-defined capsule. The cut surface was nodular and did not conform exactly to the picture of a merely hyperplastic lymph node. Microscopic examination revealed a lymph node, largely occupied by tubercle-like lesions composed of epithelioid cells among which there were small giant cells, not arranged in typical tubercles. At no place could

Fig. 1.

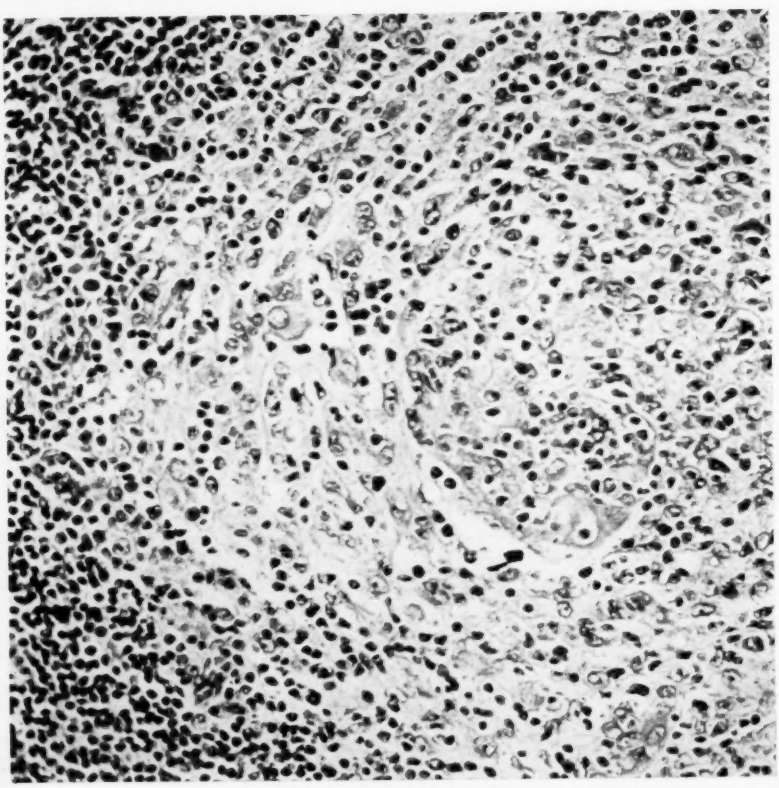

Fig. 2.

caseation be found. Minute foci of necrosis were present but extended search resulted in failure to demonstrate any acid-fast bacilli. The changes, on the other hand, were typical of those found in Boeck's sarcoidosis (Fig. 1). These findings stimulated roentgenologic examinations of the chest and of the bones of the hands and feet, yet no evidence of generalized Boeck's sarcoidosis could be found. The skin reaction to tuberculin in a dilution of 1:1,000 was negative, and there was never any drainage from the biopsy incision. Finally, the tonsils removed five years previously were re-examined in a series of sections, and a single sarcoid in one tonsil was found which was not evident in the routine sections (Fig. 2). Unfortunately we cannot say whether or not this is the tonsil from the same side as the excised lymph node from the neck.

Fig. 3.

CASE 2.—J. C., a 27-year-old white man, a member of the Kingston Fire Department, had noted a swelling of the right axillary region for six months; the mass was not painful. He was never "feverish" and had not lost weight. On November 15, 1939, a mass, 6 by 5 by 3 cm., was excised as well as three smaller oval structures resembling lymph nodes. All of these masses were well encapsulated and upon gross section showed slightly yellowish, minutely lobulated cut surfaces without evidence of softening or caseation. Microscopically, numerous lesions resembling so-called "hard tubercles" were found, and these consisted of large epithelioid cells in clusters but were devoid of typical Langhan's cells or caseation which characterize true tubercles. A few of these lesions did contain giant cells. No acid-fast bacilli could be found in the lesions, but in spite of these observations recorded at the time of the original examination, a diagnosis of tuberculous lymphadenitis was made. Not until one year later was the diagnosis questioned by the attending surgeon, who failed to observe other clinical signs or symptoms of an acid-fast infection. Upon re-examination of the sections in the light of recently acquired experience with the lesions of Boeck's sarcoidosis, it became clear at once that the original diagnosis was in error and that the changes were typical of the latter condition (Fig. 3).

Subsequent to the submission of a revised pathologic report, roentgenologic examination of the chest and of the bones of the hands and feet failed to demonstrate any changes, including those of generalized Boeck's sarcoidosis, and intradermal tuberculin in a dilution of 1:1,000 gave no reaction.

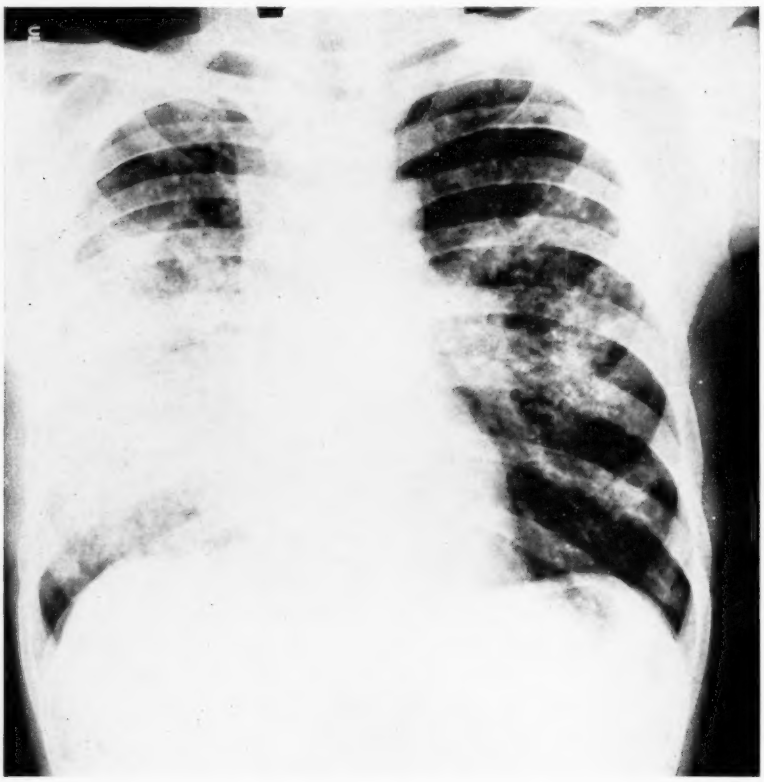

Fig. 4.

CASE 3.—G. E., a colored man, 31 years old, had been in excellent health all his life except for the usual childhood diseases until he became aware of an increasing dyspnea upon exertion early in January, 1935. Finally the dyspnea became so marked when at rest that it was necessary to hospitalize him on January 16. Upon admission to the Kingston Hospital the positive findings were those of marked dyspnea while lying in bed, pain and tenderness over the abdomen, more particularly in the gall bladder region, and changes in the lungs revealed only by roentgenograms. These latter changes were thought to be the result of either bronchopneumonia or miliary tuberculosis, in spite of normal temperature, no increase in the pulse rate, and the failure to demonstrate specific incitant organisms in the sputum after exhaustive search. There was a normal blood picture with perhaps a slight relative lymphocytosis. During two weeks' stay in the hospital he was afebrile, the pain in the abdomen subsided, and the dyspnea was relieved. A few days after discharge the pains recurred and were accompanied by "indigestion." The liver was now slightly enlarged and exquisitely tender. Upon readmission to the hospital the temperature was 100.2° F. and the pulse rate had increased to 108. No evidence of disease of the gall bladder and gastrointestinal tract was shown by x-ray studies and many other laboratory procedures. He became more comfortable upon the administration of sedatives and was discharged a few days later with a diagnosis of hepatitis.

During another admission in March of this same year an exploratory laparotomy yielded no additional information. The liver was large and bluish gray, and the gall bladder was

drained. Recovery from the operation was uneventful, but because of persistent changes in the lungs suggestive of tuberculosis, the patient was referred to the out-patient department of the Ulster County Tuberculosis Hospital. Later, owing to the difficulty in confirming a diagnosis of tuberculosis, he was admitted to this hospital for more intensive study. The observations of Dr. George Weber were most interesting: "The physical findings and x-ray plates (Fig. 4) seem to show a tuberculous process of the caseous-pneumonic type, involving chiefly the middle and lower lobes of the right lung with some spread to the left side. An enlarged mediastinal shadow, which looks like tumor growth, cannot be explained on the basis of tuberculous involvement in an adult." The sputum never yielded acid-fast bacilli, and several guinea pigs inoculated with sputum remained well. The general condition of the patient was always good, and there was a steady gain in weight. Upon discharge from the Tuberculosis Hospital, Dr. Weber further recorded that there was grave doubt of the diagnosis of pulmonary tuberculosis, but no other disease process suggested itself.

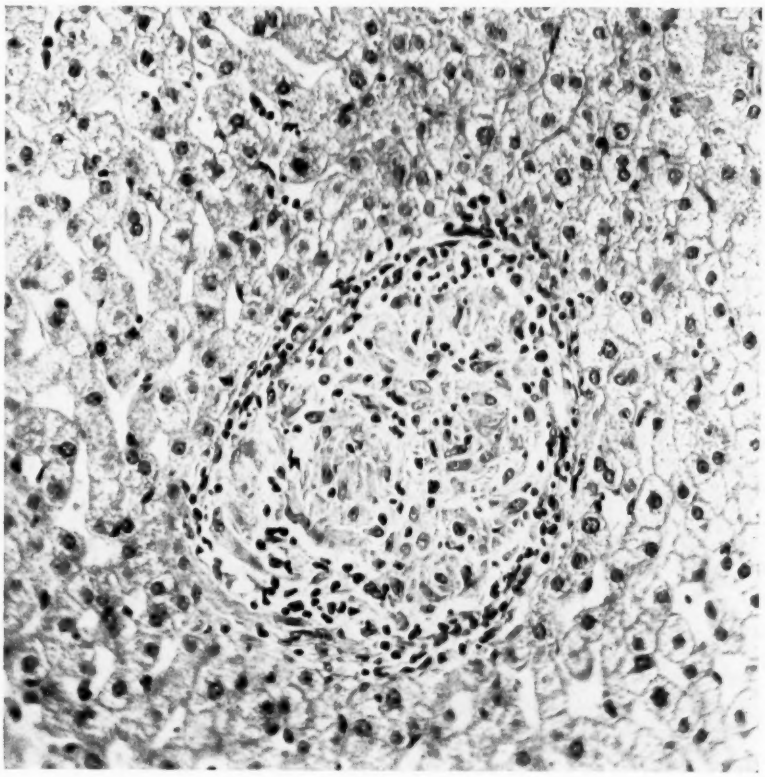

Fig. 5.

About the time the patient left this hospital, enlargement of lymph nodes over the body began and they increased in size rapidly. This lymphadenopathy was accompanied by the loss of 30 pounds in weight. One node was removed from the inguinal region on October 16, 1935, measuring 10 cm. in its greatest dimension. Enlarged glands were also present in the neck, postauricular and epitrochlear regions, and in the axillae. Histopathologic studies revealed lesions which resembled tubercles of the so-called "hard" variety in which no acid-fast bacilli could be found after extensive search. In spite of this failure to find the organisms a diagnosis of tuberculous lymphadenitis was submitted.

Because of the loss of weight, glandular enlargement, and a histologic diagnosis of tuberculous lymphadenopathy, the patient was kept in the out-patient clinic at the Tuberculosis Hospital.

Early in January, 1936, acute abdominal pain and distress began and increased in severity over a period of four weeks, after which time a second laparotomy was done at the Kingston Hospital. The liver this time was found to be far larger than upon the previous laparotomy, and its edge extended 10 to 12 cm. below the costal margin. It was studded with innumerable grayish-white nodules, most of which were only a few millimeters in diameter. Great enlargement of the perigastric lymph nodes was also seen. One of these lymph nodes and a small piece of liver containing one of the small nodules were removed for microscopic examination. Again the histologic diagnosis was tuberculosis involving the liver (Fig. 5) and perigastric nodes (Fig. 6), even though no acid-fast bacilli could be found in the lesions and macerated tissue injected into guinea pigs produced no disease.

Fig. 6.

Following this last operation and without specific therapy of any sort, the patient began to improve rapidly. During the period from February 16, 1936, to August 16, 1940, the clinical manifestations of the disease practically disappeared. He has been working for almost five years at his regular occupation as day laborer and feels entirely well. Early in August, 1940, inoculations of old tuberculin were done intracutaneously for the first time, in dilutions varying from 1:100,000 to 1:100, and yielded questionable reactions only in the lowest dilution. In June, 1941, small lymph nodes, barely palpable, were the only residual manifestation of the disease, while roentgenograms revealed complete resolution of the pulmonary and mediastinal changes previously observed, and no changes in the bones of the hands and feet could be demonstrated. Chemical examinations of the blood showed normal levels for calcium, cholesterol, total protein, and an albumin-globulin ratio of 2.75. No Bence-Jones protein was ever found in the urine.

COMMENT

Although Hutchinson, Besnier, and Boeck described this malady as a dermatologic affection, it became evident to subsequent investigators that the cutaneous manifestations were merely part of a diffuse systemic disease of a granulomatous nature. Moreover, it is now clear that this skin eruption not only may not be prominent but often does not appear at all. Schaumann[11] in 1914 mentioned the possibility of the disease pursuing a concealed existence in the lymphatic and internal organs without giving rise to skin lesions, and that in all probability the skin was attacked in comparatively rare instances. Our cases demonstrate a predilection for the lymphatic system without skin manifestations and afford additional evidence of the validity of Schaumann's original assumption.

In the light of present knowledge it is difficult to accept our first two cases as instances of isolated glandular involvement. With the limited clinical means of exploring the body, obscure involvement of other lymphatic structures might well have been present. Although a thorough clinical examination of the peripheral glands and radiographic studies of the chest revealed no evidence of adenopathy, it has been pointed out that histologic examination of the clinically normal appearing lymphatic structures will often reveal sarcoids. Thus in Case 1, only after re-examination of a series of sections of tonsils removed five years previously was a single miliary sarcoid demonstrated. Unfortunately, since no further studies were permitted, we were unable to observe the histology of nonpalpable glands or tonsils of Case 2.

The third case presented many signs and symptoms of a disseminated tuberculous process. There were slight fever with cough, weight loss, enlargement of the cervical, axillary, inguinal, mediastinal, and mesenteric lymph nodes, as well as pulmonary parenchymal infiltration of a caseous pneumonic type, and marked enlargement of the hilar shadows. Lesions in the liver and perigastric lymph nodes were found which resembled tubercles of the "hard" variety. Skin manifestations were absent, the tuberculin reactions were not significant, and tubercle bacilli could not be found in the sputum or excised tissues. The subsequent clinical course proved the diagnosis of miliary tuberculosis to be incorrect, and re-examination of the extirpated glands established the true diagnosis. Since generalized Boeck's sarcoidosis is usually a benign, nonfatal disease, there is no doubt that many reported cures of miliary tuberculosis are in fact instances of recovered sarcoidosis.

In this connection it is recalled with a great deal of interest by one of us how Dr. William S. Thayer at one time was thoroughly convinced that he had observed a case of miliary tuberculosis which eventually recovered. A biopsy of the skin in his case revealed lesions which he and the pathologist interpreted as tubercles, but in which no acid-fast bacilli could be found. He was never quite satisfied that exhaustive search might not have revealed the presence of tubercle bacilli, and often regretted that animal inoculation had been rendered useless because of the fixation of the specimen before it reached the pathologic laboratory.

The true nature of the lesions has not been appreciated by many trained observers even though numerous descriptions of the histologic pathology have been recorded. Boeck mentioned sharply circumscribed foci of "new growth" composed of epithelioid connective tissue cells with giant cells of "sarcomatous" nature. Schaumann[12] in 1924 characterized the lesions as sharply circumscribed epithelioid tubercles without necrosis and very little lymphoid reaction. Nickerson[13] believes the following findings are of assistance in differentiating sarcoid from tuberculosis: (1) Caseation is never present. (2) The giant cells are different from those seen in tuberculosis. (3) Lesions in tissues other than the lungs do not contain carbon pigment. (4) In the liver the lesions are most numerous in the portal triads, with only a few in the mid-zones of the lobules.

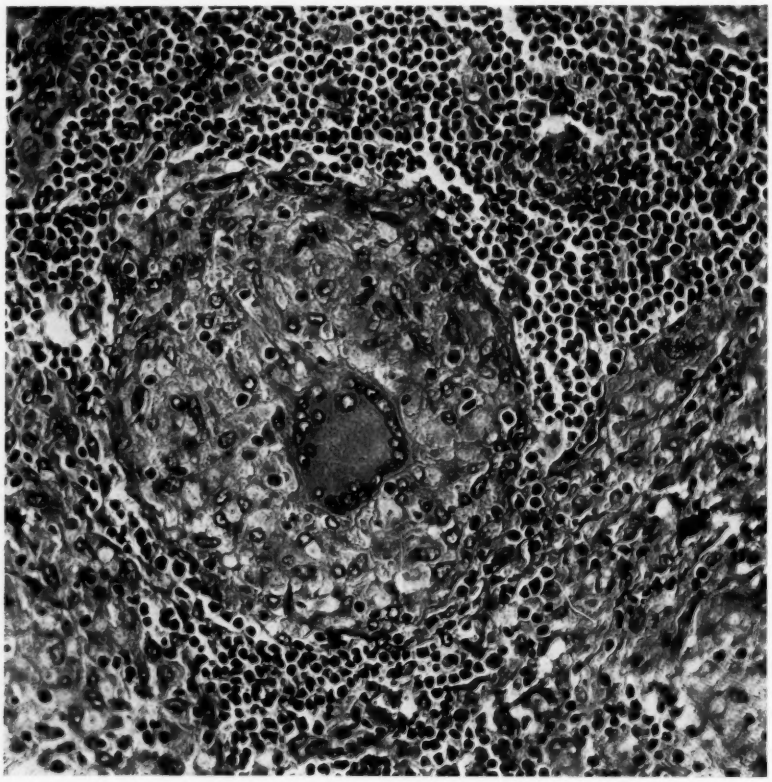

Fig. 7.

In miliary tuberculosis the reverse is true. (5) With silver impregnation, delicate reticulum is always present in the lesion. In tuberculosis this is destroyed by the onset of caseation.

In our experience we have found it exceedingly difficult to differentiate sarcoidosis from truly noncaseating tuberculosis which even to the present time are not considered separate entities by some careful students of tuberculosis (Pinner[14]). However, recently our studies on the cases herein reported have convinced us that sarcoidosis is not an unusual form of tuberculosis but rather a distinct disease entity whose etiology is still obscure. The reasoning of Pinner

is interesting but not convincing. Often the problem confronting the pathologist is impossible of solution by mere histologic examination, yet there is a pattern presented by the sarcoid lesions to one who is familiar with them that enables him to distinguish the sarcoid from the noncaseating tubercle. Every student of sarcoidosis has recognized the absence of caseation and the slight peripheral lymphocytic reaction about the sarcoids, yet in a given true noncaseating tubercle a similar situation often obtains. Each attempt thus far to point out the distinguishing features between the sarcoid and the noncaseating tubercle has failed to convey in words what may readily be seen in sections. Our attempt perhaps also is futile, but it seems to us that the epithelioid cells in the individual sarcoid are usually larger and more cuboidal and that the individual sarcoids do not fuse to produce a conglomerate sarcoid in the manner that primary tubercles produce the conglomerate tubercle. The size of the giant cell, as well as the position and number of their nuclei, throw little light on the diagnosis. It can be seen in Fig. 7 that typical Langhan's giant cells may be present.

We urge those pathologists who do not yet recognize these sarcoid lesions to study all their cases of noncaseating tuberculosis with this disease in mind. Doubtless many clinicians, as well as pathologists, will need to observe a recovered case of "miliary tuberculosis," as was true with us, before they are convinced that the clinical-histologic picture is not that of tuberculosis but instead that of sarcoidosis.

We acknowledge our indebtedness to Doctors Fred S. Carr, Douw S. Meyers, and Frank A. Johnston for the privilege of studying these cases with them. The clinical observations herein recorded are essentially those made by these respective physicians. The photomicrographs were made by Mr. Lionel B. Herrington.

REFERENCES

1. Hutchinson, J.: Cases of Mortimer's Malady, Arch. Surg. **9**: 307, 1898.
2. Besnier, E.: Lupus pernio de la face, Ann. de Derm. **10**: 333, 1889. (Quoted by Hunter.)
3. Boeck, C.: Multiple Benign Sarkoid of the Skin, J. Cut. & G. U. Dis. **17**: 543, 1899.
4. Kuznitzky, E., and Bittorf, A.: Boecksches Sarkoid mit Betheiligung innerer Organe, München. med. Wchnschr. **62**: 1349, 1915.
5. Kienbock, R.: Zur Radiographischen Anatomie und Klinik der Syphilitischen Knochenerkrankungen an Extremitäten, Ztschr. f. Heilk. (Chir.) **23**: 130, 1902.
6. Jüngling, O.: Ostitis Tuberculose Multiplex Cystica (eine eigenartige Form der Knochentuberculose), Fortschr. a. d. Geb. d. Röntgenstrahlen **27**: 375, 1919-1921.
7. Harrell, George: Generalized Sarcoidosis of Boeck: Clinical Review of Eleven Cases, With Studies of the Blood and the Etiologic Factors, Arch. Int. Med. **65**: 1003, 1940.
8. Spencer, J., and Warren, S.: Boeck's Sarcoid: Report of a Case, With Clinical Diagnosis Confirmed at Autopsy, Arch. Int. Med. **62**: 285, 1938.
9. Tice, F., and Sweany, H.: A Fatal Case of Besnier-Boeck-Schaumann's Disease With Autopsy Findings, Ann. Int. Med. **15**: 597, 1941.
10. Longcope, W., and Pierson, J. W.: Boeck's Sarcoid (Sarcoidosis), Bull. Johns Hopkins Hosp. **60**: 223, 1937.
11. Schaumann, J.: Lymphogranulomatosis Benigna in the Light of Prolonged Clinical Observations and Autopsy Findings, Brit. J. Dermat. **48**: 399, 1936.
12. Schaumann, J.: Benign Lymphogranuloma and Its Cutaneous Manifestations, Brit. J. Dermat. **36**: 515, 1924.
13. Nickerson, D. A.: Boeck's Sarcoid—Report of Six Cases in Which Autopsies Were Made, Arch. Path. **24**: 19, 1937.
14. Pinner, M.: Noncaseating Tuberculosis, Am. Rev. Tubere. **37**: 690, 1938.
15. Hunter, F. T.: Hutchinson-Boeck's Disease (Generalized "Sarcoidosis"), New England J. Med. **214**: 346, 1936.

CLINICAL CHEMISTRY

CLINICAL STUDIES WITH THE AID OF RADIO-PHOSPHORUS*

V. Early Effects of Small Amounts of Radio-Phosphorus on Blood Cell Levels, Uptake, and Excretion

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IT IS KNOWN that radioactive phosphorus emits β rays. Its half life is 14.3 days. When radio-phosphorus is administered orally or intravenously it has been found to localize in certain tissues of the body, namely, bone, bone marrow, neoplastic and leucemic tissue.[1] For this reason, radio-phosphorus has been used as a therapeutic agent in the treatment of leucemia, polycythemia vera, and lymphosarcoma. Extensive studies concerning the early absorption and distribution in blood and the early excretion in urine of therapeutic doses of radio-phosphorus in leucemia and polycythemia vera and in normal persons have been carried out.[2] These investigations have established definite patterns of response in the three groups in question when moderate-sized therapeutic doses are used. No standardization by means of very small doses has heretofore been attempted nor have any studies, so far as we are aware, been directed to the early effects of radio-phosphorus on blood cell levels in any of the three groups.

It is known that x-rays even in small doses produce biological effects long before therapeutic responses can be observed.[3] Such effects occur within the first twenty-four hours and concern fluctuations in peripheral blood levels, of red and white cells, reticulocytes, and platelets, and the relationships of cells in the differential count. It has been our purpose in the present study to determine whether internal radiation resulting from intravenous administration of radio-phosphorus in small doses elicits similar responses. We have been concerned also with the early absorption and distribution in the blood of such doses, and with the rate of excretion in the urine. Attention has been given to the influence of previous x-radiation on the early response to radio-phosphorus, and we have attempted to differentiate between the effect of inert phosphorus and its radioactive isotope. Finally, we have observed the spontaneous fluctuations of blood cell levels over a period of three hours in some of the cases here presented.

The dose chosen was approximately 500 microcuries. Assuming a total body weight of 60 to 65 kg. and an even distribution of the radioactive metabolite, there would be 0.006 to 0.009 microcuries P^{32} per gram of body weight. We have arbitrarily called this a "threshold dose" for two reasons: First, in order

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to distinguish it from tracer doses used in animal experiments[4]; and second, because the biological effects are transitory and thus differ from so-called therapeutic effects.

The subjects have included apparently healthy persons and patients with various types of leucemia and with polycythemia vera.

METHODS

Radioactive phosphorus was produced in the 60-inch cyclotron[5] by the bombardment of red phosphorus which was then transformed into isotonic Na_2HPO_4 solution containing 15 mg. of the compound per cubic centimeter. Subjects came to the laboratory in a fasting condition. Prior to the injection a blood count was made. This was repeated hourly for the first three hours after injection and at twenty-four hours. Withdrawal of 15 to 20 c.c. of blood by venipuncture was done at hourly intervals for the first three hours after injection and at twenty-four hours for the normal persons and for some of the patients. In one group of cases urine was collected at hourly intervals during the first three hours and at twenty-four hours.

One group of three leucemia patients prior to receiving any radio-phosphorus were given intravenously 3 c.c. (45 mg.) of inert Na_2HPO_4. Counts were made on these patients prior to injection and at hourly intervals for the first three hours and at twenty-four hours. A second group of patients was observed over a period of three hours for spontaneous fluctuations in blood cell levels.

The counts included: hemoglobin by the Osgood-Sahli method; red and white cell counts; platelets and reticulocytes by the cover slip method, using 1 per cent brilliant cresyl blue in 3.8 per cent sodium citrate. Smears were stained by the Wright-Giemsa technique, and at least 200 cells were enumerated for each differential count. The blood withdrawn from the veins was heparinized, cooled, and centrifuged for twenty minutes at 1,450 $\times$ gravity to insure constant volume. The plasma was removed, the buffy coat was aspirated, suspended in Riger's solution, and centrifuged again for twenty minutes at 1,450 $\times$ gravity.[2a] Finally the red blood cells were aspirated.

Materials for radioactive assay were measured, dried at 90° C., and assayed in a standard DuBridge electrometer and compared with a uranium standard. The amounts measured did not exceed 20 to 30 mg. per square centimeter, and, therefore, no correction for β ray absorption is necessary.

RESULTS

Spontaneous fluctuations were negligible for all aspects of the blood count for each of the three patients observed. These included one case of chronic myeloid and two cases of chronic lymphoid leucemia. The other results are summarized in Tables I, II, and III.

The effects of inert Na_2HPO_4 on two patients with chronic lymphoid leucemia and one with chronic myeloid leucemia are shown in Table I. The case of F. B. was characterized by only very slight elevation of the total white blood cell count, but this was made up almost entirely of lymphoid cells many of which were immature forms. The platelet count was very low from the outset.

TABLE I

CHANGES IN BLOOD CELL LEVELS FOLLOWING ADMINISTRATION OF INERT Na_2HPO_4

CASE	HB. %					R.B.C. MILLIONS					W.B.C. THOUSANDS					PLATELETS THOUSANDS					RETICULOCYTES %				
	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.
4 Case F. B. Lymphoid leucemia	109	109	109	104	-	5.7	5.7	5.1	5.4	-	15.7	14.9	14.4	13.0	-	45.6	45.6	40.8	64.8	-	1.2	1.2	1.0	1.0	-
10 Case P. P. Myeloid leucemia	73	76	73	72	74	3.9	4.3	-	3.7	3.9	118.6	90.0	134.0	124.2	120.0	128.0	340.0	970.0	840.0	871.0	0.8	0.6	0.6	0.6	0.6
5 Case L. C. Lymphoid leucemia	95	95	89	82	-	4.8	4.5	4.7	5.1	-	113.1	102.4	113.5	102.5	-	117.2	244.0	397.0	171.5	-	1.0	1.2	1.2	1.0	-

None of the elements of the blood count exhibited any significant change. The case of chronic myeloid leucemia, which had been treated with x-rays up to three weeks before admission, exhibited some lability of the white blood cell count, possibly attributable to x-rays.

Cases 1, 2, and 3 of Table II show the typical hematologic response to 500 microcuries of P^{32} in three healthy persons. In all these subjects the platelets show marked deviations from the original levels during the first three hours. The shapes of the curves are the same in all three cases. There is a deviation beyond physiologic limits in the red blood cell count and hemoglobin, and this is closely correlated with the response of the reticulocytes. The total white blood cell count in all these cases remained unaffected.

Cases 4 through 10 of Table II show hematologic responses in lymphoid and myeloid leucemia patients. There is a definite effect on all elements of the blood count in these cases with the exception of the total white blood cell count of Case 4. Here it will be noted that the initial level of the white blood cells was only slightly elevated above normal. The platelet count in this case, which was unaffected by inert Na_2HPO_4, shows a definite rise and fall similar to those of the other cases of this group. The early radiation effect on the reticulocytes is especially pronounced in Cases 7 and 9. In all cases the point at which the reticulocyte level is at maximum the platelets are at minimum.

Cases 11 through 13 of Table II show hematologic responses in polycythemia vera. The red blood cells and the hemoglobin are the elements most profoundly affected, although all elements show some lability. The deviation of the reticulocytes is downward. The platelet count is markedly changed, but there seems to be no pattern of platelet response characteristic for this condition.

Case 14 of Table II shows the hematologic response in one case of polycythemia vera with leucemoid reaction. It would appear from this case that the typical responses of leucemia and polycythemia patients are here combined, with lability of the red blood cells, white blood cells, and platelets, and the reticulocytes exhibiting an upward trend.

Cases 1, 2, and 3 of Table III illustrate rates of uptake in three normal persons. The maximum excretion is approximately the same in all cases and is reached in all within the first three hours. Case 2 exhibits a somewhat higher uptake by white blood cells than the other two cases in this group. This may well be correlated with the somewhat higher level of the total white blood cell count in this person.

Cases 4, 5, 7, 9, 10 of Table III illustrate rates of uptake in leucemia patients. In Case 5 the utilization of phosphorus by white blood cells is in keeping with the excessive white blood cell proliferation as opposed to Case 4 where the total white blood cell count was never higher than 20,000. Case 9 shows a strikingly high uptake of phosphorus by white blood cells, despite the relatively low total white blood cell count. This may be explained on the basis that the differential count in this case consistently showed 85 to 90 per cent myeloblasts. It has been shown by Marshak[6] that cells in the early stages of mitosis are the largest consumers of phosphorus. Case 4 with a relatively low total white blood

TABLE II

CHANGES IN BLOOD CELL LEVELS FOLLOWING SMALL DOSES OF P^{32}

CASE	HB. %					R.B.C. MILLIONS					W.B.C. THOUSANDS					PLATELETS THOUSANDS					RETICULOCYTES %				
	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.	0	1 HR.	2 HR.	3 HR.	24 HR.
1. D. V. N. Normal Male 46 yr.	111	119	109	121	126	5.7	5.9	5.3	6.4	6.2	10.0	9.6	8.2	11.0	10.7	287.0	1,063.8	229.0	401.7	598.0	1.2	1.6	1.0	2.4	1.6
2. R. M. Normal Female 38 yr.	119	110	115	106	102	4.8	4.7	5.0	4.3	5.0	13.8	11.6	11.5	14.4	15.8	115.1	368.1	1,200.0	219.0	244.3	0.5	2.2	1.0	0.6	0.8
3. F. V. N. Normal Female 20 yr.	112	108	111	110	97	5.6	5.5	5.2	4.8	5.9	7.1	7.8	7.4	10.1	6.7	332.0	789.0	73.0	193.5	71.5	0.4	1.2	0.8	1.2	1.8
4. F. B. Chronic lymphoid leucemia Male 40 yr.	–	109	110	109	118	–	5.9	5.5	6.0	5.5	–	15.5	14.7	16.0	17.3	–	47.1	153.2	95.9	132.2	–	0.8	0.4	1.2	0.8
5. L. C. Chronic lymphoid leucemia Male 52 yr.	92	92	96	95	97	4.8	4.9	4.8	4.5	4.7	106.4	119.1	112.5	118.6	133.1	–	921.0	154.0	223.0	150.0	2.0	1.8	2.4	2.2	1.6
6. F. L. Chronic lymphoid leucemia Female 66 yr.	82	75	73	80	79	4.7	4.6	3.8	5.1	4.1	101.0	89.0	102.7	88.3	127.8	87.3	119.8	391.8	533.5	255.3	0.6	1.0	1.3	0.6	1.6
7. A. W. Chronic myeloid leucemia Female 12 yr.	39	39	39	41	44	2.0	2.4	2.2	2.1	2.4	390.0	387.6	419.3	355.8	400.0	240.0	547.0	196.0	125.0	561.0	0	1.5	3.6	6.4	5.4
8. C. K. Chronic myeloid leucemia Male 24 yr. Previous treatment P^{32}	83	86	86	89	85	4.8	5.2	5.6	5.0	4.4	51.5	47.9	46.8	44.3	53.2	337.4	790.1	374.0	766.0	172.0	0.7	0.7	0.7	0.3	0.1

9. O. H. Subacute myeloid leucemia Male 26 yr.	39	39	39	39	42	2.4	2.2	2.4	2.5	2.1	11.3	13.0	14.3	11.5	20.8	150.0	167.0	102.0	269.0	602.0	0	0.1	4.2	–	1.6
10. P. P. Chronic myeloid leucemia Female 32 yr. Recent treatment with x-ray	74	76	76	72	76	3.9	3.9	3.6	4.2	3.7	120.0	103.3	103.6	121.8	117.2	871.0	780.0	860.0	870.0	920.0	0.6	0.9	1.0	0.9	0.8
11. G. Polycythemia vera Male 37 yr.	128	128	128	122	125	6.4	6.9	7.0	6.8	6.8	8.2	–	10.7	8.6	5.2	2,636.0	829.0	189.0	609.0	750.0	2.0	1.6	0.7	1.2	0.9
12. F. M. Polycythemia vera Male 59 yr.	116	117	115	115	115	7.9	8.9	10.2	10.8	10.1	19.4	20.6	18.5	23.1	28.4	908.0	826.0	1,034.0	1,200.0	737.0	0.5	0.3	0	0.1	0.3
13. H. W. Polycythemia vera Male 77 yr.	90	88	79	97	88	6.6	7.3	7.5	6.4	7.8	54.4	48.1	49.4	44.3	47.3	1,400.0	973.0	2,180.0	636.0	Innumerable	2.2	1.1	0.9	0.9	0
14. H. H. Polycythemia vera with leucemoid reaction Male 65 yr. Previous treatment P^{32}	114	113	115	103	119	7.5	6.8	7.8	6.7	7.6	32.7	33.6	38.2	33.2	34.0	301.2	227.0	–	80.4	880.9	0	3.1	–	1.5	0.9

TABLE

UTILIZATION AND EXCRETION OF P^{32}

CASE	WHOLE BLOOD								R.B.C.									
	1 HR.		2 HR.		3 HR.		24 HR.		1 HR.		2 HR.		3 HR.		24 HR.		1 HR.	
	μC /c.c.	%	μC /c.c	%	μC /c.c	%	μC /c.c.	%	μC /c.c.	%	μC /c.c.	%	μC /c.c.	%	μC /c.c.	%	μC /c.c.	%
1. D. V. N. Normal Male 46 yr.	0081	1610	0094	1880	0081	1610	0061	1220	0011	0221	0106	2120	0139	2780	0131	2620	0192	3840
2. R. M. Normal Female 38 yr.	0094	1879	0090	1799	0089	1785	0212	4240	0108	2159	0134	2680	0146	2925	0119	2382	0318	6370
3. F. V. N. Normal Female 20 yr.	0125	2501	0126	2521	0187	3730	0077	1541	0113	2262	0141	2821	0120	2401	0127	2539	0165	3302
4. F. B. Chronic lymphoid leucemia Male 40 yr.	0084	1681	0072	1442	0095	1901	0043	0860	0088	1759	0106	2119	0099	1980	0059	1179	0316	6310
5. L. C. Chronic lymphoid leucemia Male 52 yr.	0084	1621	0074	1432	0063	1219	0052	0985	0084	1621	0095	1800	0095	1800	0095	1800	0286	5502
7. A. W. Chronic myeloid leucemia Female 12 yr.	0C894	1891	00824	1742	.....	.....	00282	0597	0020	0423	0082	1736	.....	.....	0062	1310	0821	1 7390
9. O. H. Subacute myeloid leucemia Male 26 yr.	0030	0636	0018	0381	0018	0381	0009	0191	0040	0884	0042	0889	0052	1010	0C36	0764	1150	2 440
10. P. P. Chronic myeloid leucemia Female 32 yr. Recent x-ray	0087	1735	0077	1541	0052	1039	0052	1039	0095	1901	0105	2010	0107	2013	0104	2080	0697	1941
11. G. Polycythemia vera Male 37 yr.	0303	4061	0309	4102	0209	2799	0172	2298	0296	3940	C362	4803	0391	5202	0302	4061	0464	6180
14. H. H. Polycythemia with leucemoid reaction Male 65 yr. Previoustreatment with P^{32}	0247	4390	0216	3839	0213	3780	0275	4700	0066	1172	0259	4699	0229	4062	0251	4449	0617	1 064

cell count shows a high level of excretion at the first hour, while the excretion rate of Case 5 with over 100,000 cells (white) reaches its peak at the third hour. The excretion of Case 7 is considerably lower than that of the normal persons or of the other patients in this group. This would seem to be in accord with the progressive uptake of phosphorus by newly forming white cells.

Case 11 of Table III shows uptake in a case of polycythemia vera. The red blood cells here show the highest uptake of phosphorus during the twenty-four-hour period of any of the groups studied. The difference between this case and the normal person in this respect, however, is very much less than the difference demonstrated between polycythemia and leucemia. The work of Erf and Lawrence[2b] indicates that the uptake of phosphorus by red blood cells is the same in normal persons and polycythemia patients at the twenty-four-hour point. These authors extended their observations beyond the first day, and their results

III

PER CENT OF DOSE PER 100 C.C.

W.B.C.						PLASMA								URINE							
2 HR.		3 HR.		24 HR.		1 HR.		2 HR.		3 HR.		24 HR.		1 HR.		2 HR.		3 HR.		24 HR.	
μC /c.c.	%	μC /c.c	%	μC /c.c	%	μC /c.c.	%	μC /c.c	%	μC /c.c	%	μC /c.c	%	μC /c.c.	%	μC /c.c	%	μC /c.c.	%	μC /c.c.	%
.0218	.4352	.0134	.2681	.0313	.6251	.0094	.1880	.0054	.1079	.0021	.0420	.0019	.0379	.0612	1.2210	.7368	14.73	1.022	20.39	.075	1.500
.0251	.5001	.0051	.1019	.0134	.2681	.0094	.1880	.0030	.0600	.0026	.0521	.0013	.0260	.0250	.5000	.8205	16.41	.1550	3.102	.1075	2.150
.0202	.4040	.0206	.4104	.0114	.2280	.0110	.2220	.0073	.1459	.0042	.0841	.0027	.0540	.6000	12.000	.9010	18.020	.9500	19.00	.2000	4.001
.0211	.4220	.0125	.2501	.0127	.2539	.0046	.0921	.0035	.0700	.0034	.0680	.0012	.0239	.0940	1.8750	.9400	18.750	1.120	22.390	.0630	1.2509
.0212	.4021	.0382	.7249	.0461	.8748	.0020	.0379	.0074	.1432	.00424	.0797	.0010	.0196	.0403	.7660	.4240	7.970	.8440	16.00	.1060	2.121
.0583	1.2310	.....	.....	.1292	2.7390	.0103	.2180	.0143	.3001	.....	.....	.0032	.0676	.....	.....	.....	.....	.....	.....	.0353	.7460
.0801	1.700	.0940	2.040	.0961	2.041	.0115	.2441	.0230	.4880	.0118	.2503	.0047	.0998	Not done							
.0087	.1735	.0061	.1221	.0230	.4590	.0063	.1261	.0418	.8360	.0031	.0620	.00146	.0291	.0230	.4590	.8350	16.690	.1460	2.910	.0170	.3381
.0433	.5770	.0238	.3181	.0161	.2149	.0204	.2721	.0204	.2721	.0091	.1211	.00290	.0388	.3290	4.371	.....	.....	.1570	2.099	.3450	4.600
.0492	.8730	.0601	1.069	.0661	1.172	.0165	.2930	.....	.....	.00851	.1511	.0119	.2105	.2110	3.741	.0340	.6049	.1945	3.4410	.0365	.6490

showed an increased uptake by red blood cells in one case of polycythemia vera at the forty-eight-hour point. It appears that no real basis of comparison exists for these findings and those of the present study for the following reasons: One point only in the uptake curves, namely, the twenty-four-hour period, is common to both investigations; the doses of radio-phosphorus (and the amounts in milligrams of salt in which they were incorporated) used by Erf and Lawrence are different for the patients and for the normal persons and in both groups the doses are considerably larger than the "threshold amounts" used in this study; finally the total number of cases observed in both studies is too small to rule out the existence of individual differences in response. The possibility that the red blood cells in polycythemia vera utilize radio-phosphorus to a greater extent than do normal red blood cells, suggests that investigations similar to those here discussed might well be extended to a larger group of

polycythemia patients, in the hope of establishing conclusively the uptake pattern in this condition. In the case here presented the excretion rate is correlated with the uptake phenomena and the curve is, therefore, lower in degree and opposite in trend from the excretion curves of healthy persons and leucemia patients.

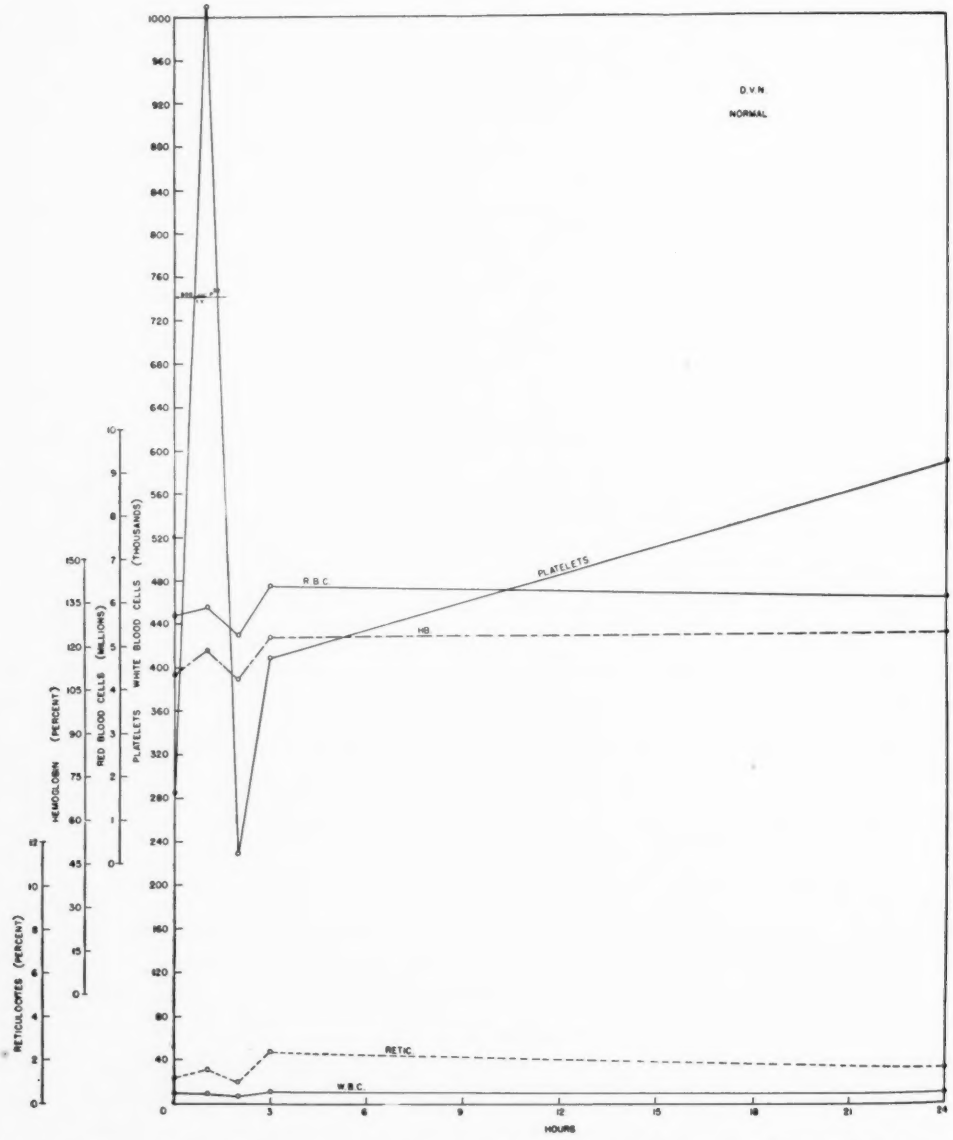

Fig. 1.—Hematologic response of normal persons to 500 microcuries P^{32} administered intravenously.

Case 14 of Table III shows uptake in the case of polycythemia with leucemoid reaction. The gradual increase in total activity of whole blood is in contrast to all other cases studied, and is no doubt accounted for by the high uptake of both red and white blood cells. The excretion curve follows those of the

leucemia patients in direction but differs in degree by reason of increased retention of radio-phosphorus by the blood.

With respect to the differential count, all the leucemia patients showed a slight increase in primitive cells (absolute numbers) during the twenty-four-hour period. This tendency did not appear in the polycythemia patients, although a few primitive cells were present in all these cases. The polycythemia patient with leucemoid reaction showed a decrease in primitive cells. There were

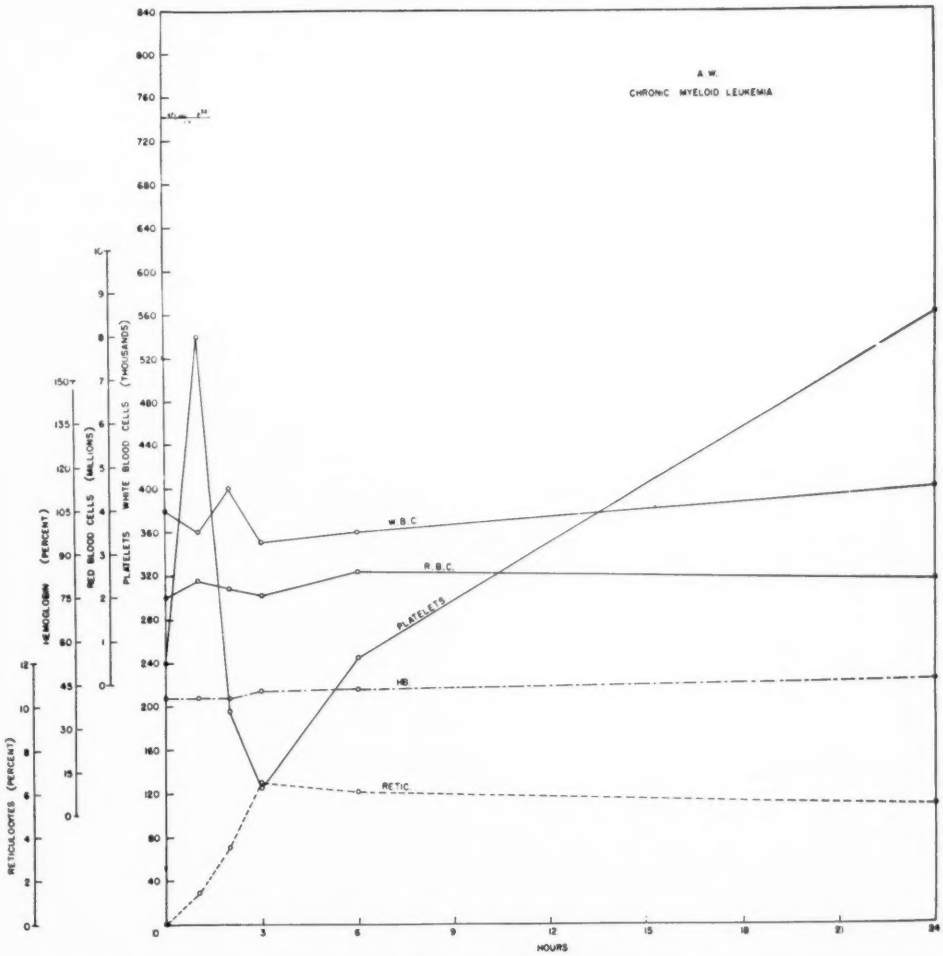

Fig. 2.—Typical early hematologic response of leucemia patients to 500 microcuries of P^{32} administered intravenously.

no shifts in the relationships of granulocytes and agranulocytes in any of the leucemia patients. The polycythemia patients showed a transitory increase in the relative number of lymphocytes during the first three hours.

The three normal persons showed no primitive cells at any time. Shifts in the relationships of other cells occurred, the most consistent being a transitory reduction in the absolute number of lymphocytes.

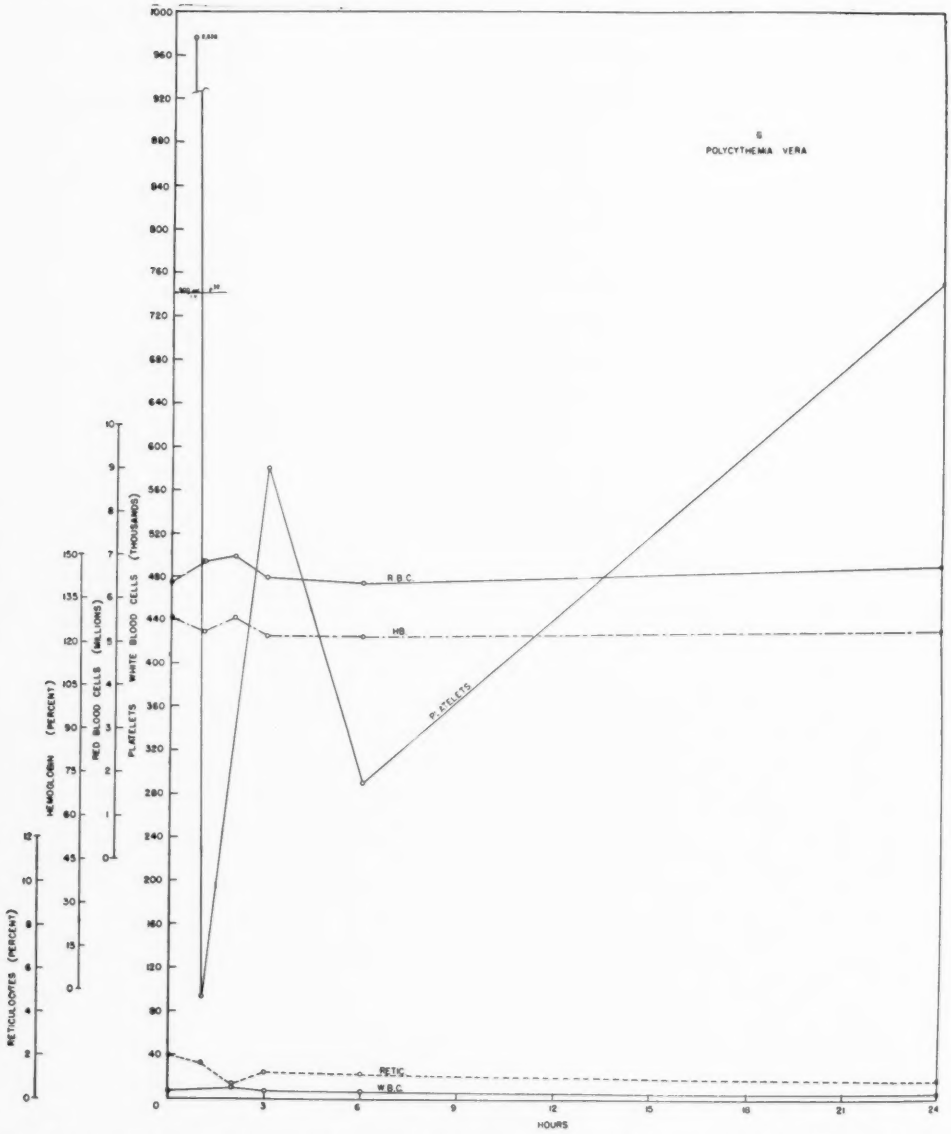

Fig. 3.—Typical early hematologic response of polycythemia vera patients to 500 microcuries of P^{32} administered intravenously.

DISCUSSION

The results show that radio-phosphorus in amounts of 0.006 to 0.009 microcuries per gram of body weight produces early radiation effects. These biological effects of very small activities of radio-phosphorus again indicate the importance of determining the "safe tracer dose" for each biological system studied.[4] The dose of radio-phosphorus used in these studies has been termed arbitrarily a "threshold dose." Experience in this study indicates that the actual threshold dose is individually conditioned by such factors as previous treatment and rate of cell production. Because of the known metabolic changes in leucemia and

polycythemia, knowledge of the basal metabolic rate might aid in predicting accurately a specific "threshold dose."

Group differences in uptake and excretion indicate the early function of the homeostatic mechanism in effecting an immediate distribution of P^{32} to the blood elements of highest metabolic rate. The excretion rate in normal persons is accordingly appreciably higher than in any of the patients.

SUMMARY

1. A threshold dose of radio-phosphorus has been arbitrarily defined.

2. Such a dose administered intravenously causes a definite "early radiation effect" on blood cell levels which is characteristic for each of the following groups: healthy persons, leucemia patients, and polycythemia patients.

3. This "early radiation effect" is further conditioned by the rate of cell production and by previous radiation treatment.

4. The uptake of P^{32} in the fractions of the blood and the rate of its excretion are roughly characteristic for each of the three groups.

We wish to express our appreciation to the staff of the Radiation Laboratory for their generous cooperation in providing the supply of radio-phosphorus used in this study.

The suggestions and criticisms of Dr. John H. Lawrence are gratefully acknowledged. One of us (B. V. A. L-B) wishes to express his appreciation for the privilege of taking part in this work at the Radiation Laboratory.

REFERENCES

1. a. Lawrence, J. H., Scott, K. G., and Tuttle, L. W.: Studies on Leukemia With the Aid of Radioactive Phosphorus, Internat. Clin. **3**: 3358, 1939.
   b. Lawrence, J. H., Scott, K. G., and Tuttle, L. W.: Studies on Neoplasms With the Aid of Radioactive Phosphorus. I. The Total Phosphorus Metabolism of Normal and Leukemic Mice, J. Clin. Investigation **19**: 267, 1940.
   c. Jones, H. B., Chaikoff, I. L., and Lawrence, J. H.: Phosphorus Metabolism of Neoplastic Tissues (Mammary Carcinoma, Lymphoma, Lymphosarcoma) as Indicated by Radioactive Phosphorus, Am. J. Cancer **40**: 235, 1940.
2. a. Erf, L. A., and Lawrence, J. H.: Clinical Studies With the Aid of Radioactive Phosphorus. II. The Absorption and Distribution of Radiophorphorus in the Blood and Its Excretion by Normal Individuals and Patients With Leukemia, J. Clin. Investigation **20**: 567, 1941.
   b. Erf, L. A., and Lawrence, J. H.: Clinical Studies With the Aid of Radioactive Phosphorus. III. The Absorption and Distribution of Radiophosphorus in the Blood of, Its Excretion by, and Its Therapeutic Effect on, Patients With Polycythemia, Ann. Int. Med. **15**: 276, 1941.
3. a. Mayneord, W. V., and Piney, E.: Some Effects of X-radiation on Blood; Measurement and Distribution of Radiation, Brit. J. Radiol. **1**: 257, 1928.
   b. Isaacs, R.: Effect of Roentgen Ray Irradiation on Red Blood Cell Production in Cancer and Leukemia, Am. J. M. Sc. **171**: 20, 1926.
   c. Spurling, R. G., and Minot, G. R.: Effect on Blood of Irradiation, Especially Short Wave Length Roentgen-ray Therapy, Am. J. M. Sc. **168**: 215, 1924.
4. Tuttle, L. W., Erf, L. A., and Lawrence, J. H.: Studies on Neoplasms With the Aid of Radioactive Phosphorus. III. The Phosphorus Metabolism of the Phospholipid, Acid Soluble and Nucleoprotein Fractions of Various Tissues of Normal and Leukemia Mice Following the Administration of "Tracer" and "Therapeutic" Doses of Radio-Phosphorus, J. Clin. Investigation **20**: 577, 1941.
5. Lawrence, E. O., and Cooksey, D.: On the Apparatus for the Multiple Acceleration of Light Ions to High Speeds, Physiol. Rev. **50**: 431, 1936.
6. Marshak, A.: Uptake of Radioactive Phosphorus by Nuclei of Liver and Tumors, Science **92**: 460, 1940.

CHANGES IN THE CREATININE CLEARANCE AND URINE FLOW OF THE DOG DURING FEVER*

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IN 1931 Goldring[1] reported that the urea clearances of human patients were usually higher in the acute febrile stage of rheumatic infection than the highest observed normal. He interpreted this as indicating a state of renal hyperfunction during the acute stage as a response to the demand of increased protein catabolism. In 1934 Grant and Medes[2] reported that the creatinine clearances of a large number of human patients with fever caused by infection were considerably higher when the body temperature was 38.9° C. or more, than when the temperature was between 36.7° and 37.1° C.

Grant and Medes[2] also reported that the creatinine clearance of normal unanesthetized dogs was increased after elevation of the body temperature by heating with conventional diathermy. But in the same year Page[3] reported that no significant change was observed in the urea clearances or in the urine flow of normal human subjects and patients with kidney disease during or after conventional diathermy. Farr and Moen[4] reported that the average urea clearance of rheumatic patients, placed in a cabinet heated by carbon filament lamps, with only the head and neck protruding, was 62 per cent of normal while the body temperature was rising and 75 per cent of normal when the body temperature was at a maximum of 104.8° to 106.7° F. Severe oliguria occurred in two experiments even though large quantities of water was given orally.

A new method of producing heat in tissues by high-frequency electromagnetic induction was introduced in 1934 by Merriman, Holmquest, and Osborne.[5] Mortimer and Osborne[6] found that during elevation of the body temperature of anesthetized dogs by this method, with the coil about the thorax, there existed a definite thermal gradient from the skin, which was heated to the highest temperature to the deeper tissues. The temperature of organs lying outside the short-wave field was elevated to a lesser degree. After heat induction was discontinued, the rectal temperature continued to rise for a short time while other parts of the body lost heat, showing that the heat was widely distributed by the circulation of the blood. Karr and Nassett[7] have elevated the body temperature of dogs, anesthetized with sodium amytal, to as much as 42.8° C. over a period of six hours by electromagnetic induction, during which time urine was collected by means of ureteral cannulae in hourly periods. They reported a decrease in total nitrogen excretion roughly parallel to the decrease in rate of urine

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flow, and found that oliguria and anuria were almost invariable sequelae to hyperthermia carried to 42° C. or higher. The blood pressure of their animals remained at a relatively high level until just prior to their death.

In 1938 Chasis, Ranges, Goldring, and Smith[8] reported that hyperemia of the kidneys occurred in human subjects during typhoid vaccine pyrexia. In the typical example they present, intravenous injection of 75 million organisms was followed in a few minutes by chills and a slight fall in body temperature, with a slight decrease in the inulin clearance. Within half an hour the inulin clearance increased about 10 per cent above normal and remained at that level, while the body temperature slowly increased over a period of six hours to a maximum of 6° F. above normal. Smith and his associates[9] have produced hyperemia of the kidneys of human beings by the intravenous injection of pyrogenic inulin. In the typical example presented, the body temperature began to increase slowly about an hour after administration of 80 mg. of pyrogenic inulin, and thirty minutes later the renal blood flow increased about 40 per cent and the inulin clearance increased about 50 per cent. The renal blood flow remained 40 to 50 per cent above the control level, and the inulin clearance varied between 10 and 50 per cent above the control level, while the body temperature increased slowly to a maximum of 4° above normal in a period of three hours.

This paper is a report of the effect of body temperature elevation upon the plasma creatinine clearance in unanesthetized dogs. The hyperthermia was produced by high-frequency electromagnetic induction, or by conventional diathermy, or by the intravenous injection of typhoid-paratyphoid vaccine, or by exposure to a current of cold air in a cold room.

EXPERIMENTAL PROCEDURE

Female dogs, trained to lie quietly during catherization, vascular puncture, and heating, were used in the postabsorptive condition, sixteen to twenty-four hours after the last meal. Water was given by stomach tube in three or four doses before the first collection period in order to produce a urine flow of 2 to 5 c.c. per minute, and enough creatinine was given orally in order that the plasma creatinine should seldom fall below 10 mg. per 100 c.c. The bladder was washed twice with 20 c.c. of sterile 1 per cent sodium chloride solution before the first and at the end of each collection period. Approximately in the middle of each period enough blood was drawn to yield 1 c.c. of plasma for analysis. The plasma proteins were precipitated by means of the acid cadmium sulfate method of Miller and Van Slyke.[10] Creatinine was determined in the appropriately diluted urines and plasma filtrates by adaptation of the alkaline picrate colorimetric method[11] to the Evelyn photoelectric colorimeter,[12] with the following procedure:

Four cubic centimeters of a solution containing between 1.600 and 0.080 mg. per 100 c.c. of creatinine are pipetted into a dry photoelectric colorimeter tube. Into another such tube are put 4 c.c. of water, to be used as a blank. With each are mixed 2 c.c. of alkaline picrate reagent. After fifteen minutes the galvanometer is adjusted to read 100 with the tube of blank in the photoelectric colorimeter, using the No. 520 color filter and the No. 6 diaphragm. The blank is then replaced by the tube of unknown and the new galvanometer reading is recorded. From a table of known concentrations of creatinine and corresponding galvanometer readings, the creatinine content of the unknown is found.

Rectal temperature was measured at frequent intervals with a standard clinical thermometer. In these experiments fever is reported in degrees and tenths of a degree of temperature Fahrenheit above the rectal temperature of the dog during the control clearance periods.

High-frequency electromagnetic induction was accomplished with a Burdick SWD-10 Triplex. The electric current, with a frequency of 12 million cycles per second, was conducted through a flexible, heavily-insulated cable, supported by hard rubber clips in the form of a helix of three turns about the part of the body to be heated, from which it was separated by additional insulation of four layers of bath toweling. When heat was induced in the head of a dog, the helix was just posterior to the eyes and about the posterior part of the lower mandible. When heat was induced in the region of the kidneys, the helix was obliquely about the caudal three or four thoracic vertebrae and the anterior abdominal region. When heat was induced in the region of the pelvis, the helix was obliquely about the sacral vertebrae and the middle abdominal region. Two or three control clearance periods preceded a twenty-minute period of heat induction, immediately after which the cable and toweling were removed from the dog to allow the body temperature to return to normal, during which time there were one to six clearance periods. The controls of the apparatus were set to give approximately the same power output in each experiment. In two experiments a fever of approximately three hours' duration was maintained by intermittent high-frequency electromagnetic induction, in which ten-minute periods with induction were alternated with ten-minute periods without induction. In three dogs the lumbar sympathetic chains and the first sacral ganglia were excised and the splanchnic nerves were sectioned in two operations upon each dog. In two other dogs the kidneys only were denervated. When the wounds had healed, heat was induced for twenty minutes in the kidney region in two, three, or four experiments with each dog. In two electromagnetic induction experiments upon a normal dog and in one experiment upon a dog with lumbar sympathectomy, the diodrast clearance was used as a measure of renal plasma flow.[13] The analytical technique of White and Rolf[14] was used in the determination of diodrast iodine in urines and plasma filtrates.

Conventional diathermy was accomplished with equipment which included a Victor spark-gap diathermy machine, made by the General Electric X-ray Corporation, and a pair of pad electrodes, each of which was a thin sheet of lead, 4½ by 8½ inches in size, covered by several layers of surgical gauze. An electrode was held tightly against each of the shaved sides of a dog between the foreleg and the flank with a pad of toweling, about 1 inch thick 6 inches wide and 12 inches long, with several strips of water-proof adhesive tape completely encircling the pads and body of the animal. The electrodes and pads were kept wet with a 1 per cent sodium chloride solution, and a high-frequency alternating current of 1,500 milliamperes was allowed to flow through the body of the dog for one to three hours. There were clearance periods before, during, and after diathermy.

After two or three control clearance periods typhoid vaccine pyrexia was produced by the intravenous injection of doses of typhoid-paratyphoid vaccine

containing from 25 million to 2 billion killed organisms. The vaccine was obtained from the Wisconsin State Laboratory of Hygiene.

Two experiments were made with a dog, in each of which, after three control clearance periods in the moderately warm laboratory, the carriage supporting the animal was wheeled into a cold room and a current of cold air was blown against the shaved sides and abdomen by means of a large electric fan. After about one hundred minutes of such exposure the carriage was wheeled back into the laboratory and clearance periods were continued for about one hundred more minutes.

RESULTS

High-frequency electromagnetic induction in any one of three body regions caused a rapid and continuous elevation of the rectal temperature, which was accompanied by vigorous panting. In a series of 18 comparative experiments with three dogs the average maximum hyperthermia observed at the end of twenty minutes was 1.0° F. when heat was induced in the head, 1.9° F. when heat was induced in the kidney region, and 3.1° F. when heat was induced in the pelvis. The temperature returned to normal in twenty to seventy minutes, and panting ceased at normal body temperature. The changes in minute volume of urine flow were inversely related to the changes in rectal temperature, as shown in Fig. 1. During maximum hyperthermia there was often an oliguria, but several minutes after the body temperature began to decrease, the rate of urine flow began to increase, until it was again between 2 and 5 c.c. per minute at normal temperature. There was no consistent relationship between the region in which heat was induced and the amount of urine flow decrease.

During the hyperthermia produced by electromagnetic induction, the plasma creatinine clearance generally decreased, but never significantly increased. In the series of 18 comparative experiments with three dogs the average maximum decrease in clearance was 13 per cent when heat was induced in the head, 45 per cent when heat was induced in the kidney region, and 27 per cent when heat was induced in the pelvis. A typical example of the results in each variety of these experiments is shown in Fig. 1, parts *A*, *B*, and *C*. When heat was induced in the kidney region in 28 experiments with nine dogs, a maximum decrease of the creatinine clearance of 12 to 79 per cent occurred twenty-two times, with a maximum hyperthermia of 0.5° to 2.6° F. In 12 of these experiments the clearance immediately increased to the control level as soon as induction was discontinued, even though the hyperthermia persisted. In eight of these experiments the clearance began to increase toward the control level as the body temperature began to decrease. In two experiments the clearance remained low. The eight experiments with no decrease in clearance during hyperthermia were with three large dogs in which it seemed impossible to produce a decrease in clearance except occasionally when the control clearances were high, i.e., more than 66 c.c. per minute.

In two experiments during electromagnetic induction of heat the plasma creatinine clearance decreased 44 and 32 per cent while the renal plasma flow decreased 32 and 35 per cent, respectively. The filtration fraction did not change significantly in either experiment. Immediately after induction was discon-

tinued, the creatinine clearance and the renal plasma flow increased again to the control values in both experiments.

The effect of electromagnetic induction after section of the splanchnic nerves and removal of the lumbar sympathetic ganglia is shown in Table I. The magnitude of decrease of the creatinine clearance during induction was much reduced as compared with that in the unoperated animal, or no decrease in the clearance occurred at all during induction after the operation.

TABLE I

EFFECT OF ELECTROMAGNETIC INDUCTION BEFORE AND AFTER SECTION OF SPLANCHNIC NERVES AND REMOVAL OF LUMBAR SYMPATHETIC GANGLIA

DOG	BEFORE SYMPATHECTOMY			AFTER SYMPATHECTOMY		
	CONTROL CLEARANCE	FEVER °F.	CLEARANCE DECREASE %	CONTROL CLEARANCE	FEVER °F.	CLEARANCE DECREASE %
S	75.0	1.0	–33	60.5	0.5	–18
	48.2	0.7	–46	57.7	0.4	–None
	53.5	1.5	–71	56.3	0.9	–20
	44.5	0.5	–71	58.5	0.9	–30
W	30.1	1.1	–66	38.7	1.4	None
	32.7	0.6	–46	38.2	1.6	None
	28.4	1.3	–35			
	32.1	1.2	–46			
T	50.8	1.3	–56	63.7	2.7	None
	47.3	2.2	–79	61.0	2.7	None
				62.7	2.2	None*

*In this experiment there was no change in the renal plasma flow during or after heating, and the filtration fraction did not change.

After denervation of the kidneys only of each of two dogs, electromagnetic induction could cause a decrease in the creatinine clearance, as shown in Table II. Apparently dog K was one of that type of dog in which a decrease in the creatinine clearance sometimes occurs during induction and sometimes does not.

TABLE II

EFFECT OF ELECTROMAGNETIC INDUCTION AFTER DENERVATION OF THE KIDNEYS ONLY

DOG	CONTROL CLEARANCE	FEVER °F.	CLEARANCE DECREASE %
SP	39.7	3.6	–49
	40.4		–52
	54.6		–82
K	70.0	2.1	None
	66.2		–24
	62.2	3.6	None

In order to make the hyperthermia produced by electromagnetic induction comparable to that of conventional diathermy both in magnitude and duration, two dogs were heated by intermittent electromagnetic induction in the kidney region for three hours. The body temperature ranged from 0.8° to 1.3° F. during this period, and the plasma creatinine clearance varied between the control level and 66 per cent of the control level. There was no increase in the clearance.

The effects of conventional diathermy upon five dogs in eight experiments were somewhat variable, as shown in Table III. In the first place, it was difficult

to induce a fever in dogs S and F, because these animals presumably were able to liberate heat very rapidly by violent panting. The other three dogs panted only gently and intermittently during diathermy, but at least an hour was necessary to produce more than a degree Fahrenheit of fever in them. There was only

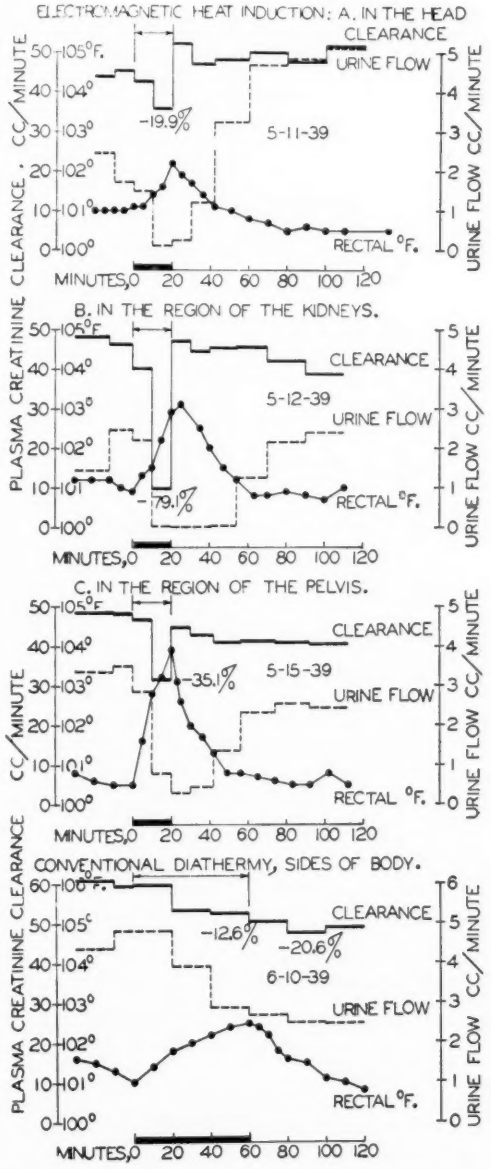

Fig. 1.—Typical changes in the plasma creatinine clearance and the minute volume of urine flow in a dog during fever produced by electromagnetic induction (*A*) in the head, (*B*) in the kidney region, and (*C*) in the region of the pelvis, and changes in clearance and urine flow in the same dog during fever produced by conventional diathermy.

a moderate, slow diminution of the minute volume of urine flow during the fever of conventional diathermy, as shown in Fig. 1. During diathermy there was either a decrease in the plasma creatinine clearance or no significant change,

except for a transitory increase of about 16 per cent in one experiment. After diathermy the clearance decreased still more in two experiments, decreased below normal in two others, and remained equal to or became equal to the controls in four.

TABLE III

PRINCIPAL CHANGES IN THE PLASMA CREATININE CLEARANCE OF DOGS DURING AND AFTER FEVER PRODUCED BY CONVENTIONAL DIATHERMY

DOG	NORMAL		HEATING PERIOD			AFTER HEATING	
	CONTROL CLEARANCE	TEMP. °F.	TIME IN MINUTES	CHANGE IN CLEARANCE %	FEVER °F.	CHANGE IN CLEARANCE %	FEVER °F.
T	60.2	101.0	60	−12.6	1.5	−20.6	0.1
R	63.3	100.9	60	−15.8	1.6	−20.5	1.1
						To normal	0.0
R	65.6	100.6	49	None	2.3	−13.3	1.4
B	44.3	100.5	59	None	1.7	None	0.2
S	63.8	101.4	78	None	0.0	−16.1	0.0
						To normal	0.0
S	49.0	100.9	110	+15.7	0.5		0.0
				To normal	0.9	None	
S	55.8	101.2	180	None	0.6	None	0.0
F	55.5	100.2	180	−11.0	0.5	To normal	0.1

The intravenous injection of typhoid-paratyphoid vaccine containing 25 million killed organisms into dog P was followed in a short time by a fever of 0.5° F., and later by an increase in the creatinine clearance of about 15 per cent. When a larger dose of 100 million killed organisms was used in each of twelve experiments with six dogs, there was no fever in two cases, but slight fever of 0.4° to 0.8° F. in ten; and no significant change in the creatinine clearance in six cases, but a moderate increase of 8 to 38 per cent in six, as shown in Table IV. In Experiments 12 and 13 an increase in the clearance was not observed until four and one-half to six hours after injection of the vaccine. When huge doses of 2 billion killed organisms were injected into dogs B and S, there was an immediate rise in rectal temperature to a maximum of more than 2° F. in about three hours. With subsequent huge doses of vaccine in dog S the onset of fever was later and the pyrexia was not so great as before. In Experiments 14, 15, and 16 the maximum clearance definitely did not coincide with the maximum fever but occurred earlier or later. In Experiments 15 and 17 the injection of the vaccine was immediately followed by an extreme, but transitory, decrease in the clearance. During all fevers by typhoid vaccine injection there was no panting and no decrease in the minute volume of urine flow other than that which normally follows a moderate diuresis.

It was found that exposure to a current of air from an electric fan in a room at 38° and 47° F. caused the dog to shiver violently and the rectal temperature to increase rapidly about a degree Fahrenheit above normal. This was followed by a decrease in the temperature to about half a degree above normal. In one experiment the plasma creatinine clearance increased to nearly 30 per cent above normal and in the other to 18.3 per cent. The minute volume of urine flow decreased only at a rate which normally follows a moderate diuresis. Upon return to the laboratory at 71° to 73° F., the rectal temperature fell to normal very quickly and the clearance decreased slowly toward the level of the controls.

TABLE IV

PRINCIPAL CHANGES IN THE PLASMA CREATININE CLEARANCE AND RECTAL TEMPERATURE OF DOGS AFTER INTRAVENOUS INJECTION OF TYPHOID-PARATYPHOID VACCINE

EXPERIMENT NO.	DOG	NORMAL		AFTER INJECTION OF KILLED ORGANISMS			
		CONTROL CLEARANCE	TEMP. °F.	DOSE IN MILLIONS	MIN. AFTER INJECTION	CHANGE IN CLEARANCE %	FEVER °F.
1	P	65.8	101.5	25	62-83	+14.8	0.5
2	P	58.8	101.1	100	136-163	None	0.5
12	P	51.0	100.6	100	267-287	+38.2	0.6
3	F	57.2	100.5	100	0-171	None	None
4	F	56.5	100.9	100	148-197	None	0.4
13	F	53.2	100.5	100	364-384	+14.3	0.4
5	T	43.6	101.0	100	0-175	None	None
6	T	43.1	101.1	100	139-187	+ 7.9	0.8
7	R	47.8	101.7	100	87-153	None	0.4
8	R	55.1	101.0	100	160-200	None	0.8
9	IV	55.7	100.6	100	145-177	+32.1	1.0
10	B	41.1	100.7	100	128-188	+25.3	0.4
11	B	40.6	100.1	100	153-201	+13.5	0.6
14	B	38.3	100.8	2000	158-178	+12.8	2.4
					238-258	+23.5	2.1
15	S	55.8	100.8	2000	0-8	−56.8	0.4
					78-98	+28.1	1.0
					198-218	+14.1	2.8
16	S	48.8	101.0	2000	221-241	+32.0	2.1
					371-391	+47.1	1.4
17	S	62.6	101.1	2000	0-10	−69.0	0.0
					88-108	To normal	0.0
					258-278	None	1.2

DISCUSSION

The results obtained from these experiments suggest that the changes which occur in the creatinine clearance of the dog during fever or hyperthermia depend upon the causal agent. During hyperthermia produced by an external physical means, such as electromagnetic induction or conventional diathermy, there was generally a decrease in the clearance but never an increase, except in one instance when a transitory increase was followed by a decrease in the clearance. This is in agreement with the results of Farr and Moen,[4] who employed the external physical means of a heating cabinet, and also with the observation of Karr and Nassett[7] that heating with electromagnetic induction decreased the total nitrogen excretion. But these results are contrary to those obtained by Grant and Medes[2] with conventional diathermy. Inasmuch as the postabsorptive clearance of many dogs varies spontaneously from day to day, it is suggested that some of the increases in the creatinine clearance reported by Grant and Medes, which were above the control clearances determined on previous days, might have been found to be equal to or less than control clearances determined just previous to diathermy on the same day.

When fever was caused by vaccine injection or by exposure to cold, there was generally an increase in the clearance, but never a decrease, except in two

instances of transitory decreases followed by increases in the creatinine clearance to or above the control level. Both of these procedures may have produced hyperthermia by virtue of increased metabolic activity. A comparison of the results obtained with these four methods of producing hyperthermia leads to the conclusion that an increase in creatinine clearance was never due to hyperthermia itself, but rather to changes in the tissues which were responsible for the hyperthermia. As further evidence for this idea it may be noted that the maximum increase in clearance did not always coincide with the maximum body temperature, after injection of typhoid-paratyphoid vaccine. This is in agreement with the observations of Chasis, Ranges, Goldring, and Smith[8] with a human subject. Furthermore, Smith[9] shows that the inulin clearance of the human subject may increase about 50 per cent after injection of pyrogenic inulin long before the maximum body temperature is attained.

The decrease in creatinine clearance during hyperthermia produced by electromagnetic induction seems to have been due entirely to a reduction of the renal blood flow because of dilatation of blood vessels in the peripheral vascular bed. The fact that a decrease in the renal plasma flow during electromagnetic induction and an increase to the control level immediately after induction occurred simultaneously with the respective decrease and increase to the control level of the creatinine clearance, with no significant change in the filtration fraction, indicates that the decrease in renal blood flow was due either to constriction of the afferent arterioles or to extrarenal shunting of much of the blood to other parts of the body. These activities seem to be brought about in part or entirely by reflexes through the thoracolumbar outflow, because section of the splanchnic nerves and removal of the lumbar chains greatly reduced or entirely abolished the decrease in clearance and, according to one observation, the simultaneous reduction in renal blood flow. That the decrease in renal blood flow is not due to constriction of the afferent arterioles is indicated by the fact that denervation of the kidneys only did not prevent a decrease in the creatinine clearance during electromagnetic induction. The decrease in creatinine clearance during the hyperthermia produced by conventional diathermy may also have been due to a reduction of the renal blood flow because of dilatation of blood vessels in the peripheral vascular bed. It is suggested that the extreme, but transitory, decreases in clearance which occurred in the typhoid vaccine Experiments 15 and 17 of Table IV may have been due to a shunting of a large fraction of the blood from the renal arteries to the splanchnic vascular area because of a transitory shock accompanied by vasodilatation immediately following the injection of the huge dose of vaccine.

The inverse relationship between the minute volume of urine flow and the magnitude of the fever produced by electromagnetic induction was probably due to temporary water conservation and not to dehydration, because the urine flow increased soon after heating ceased. This change in the urine flow did not parallel the change in creatinine clearance throughout the duration of an experiment, nor did the amount of reduction in urine flow correspond to the amount of clearance decrease, as is shown in Fig. 1. Therefore, the regulation of the rate of urine flow seems to have been independent of the rate of glomerular

filtration. It is possible that secretion of the antidiuretic principle by the hypophysis was increased as the body temperature was rapidly elevated, causing a proportional increase in the rate of water reabsorption in the kidney tubules. Attention is called to the fact that there was neither panting nor marked reduction in the rate of urine flow when fever was produced by typhoid vaccine injection. This may indicate a relationship between the mechanisms responsible for panting and for water conservation.

SUMMARY

During the hyperthermia produced by electromagnetic induction, the plasma creatinine clearance generally decreased, but never significantly increased. Heat induced over the kidney area was most effective in lowering the clearance, less effective when induced in the pelvis, and least effective when induced in the head. When heat was induced over the kidney area in 28 experiments with nine dogs, a maximum decrease of the creatinine clearance of 12 to 79 per cent, with a maximum hyperthermia of 0.5° to 2.6° F., occurred twenty-two times. This decrease of the clearance occurred about five minutes after the initial rise in temperature, but its magnitude was not related to the magnitude of the hyperthermia. The decrease of the clearance was found to be due to a simultaneous decrease of the renal plasma flow. Denervation of the kidneys only did not prevent a decrease of the clearance during the hyperthermia, but section of the splanchnic nerves and removal of the lumbar sympathetic ganglia nearly or completely prohibited this decrease of the plasma creatinine clearance.

An increase in body temperature of 0.5° to 2.3° F., caused by conventional diathermy, resulted in no significant change in the clearance in two experiments and a decrease of 11 to 21 per cent in five experiments. In one other experiment the clearance increased 16 per cent during the initial hyperthermia, but became normal again, even though the hyperthermia was maximum.

The intravenous injection of typhoid-paratyphoid vaccine resulted in a maximum fever of 0.4° to 2.8° in 15 experiments performed on seven dogs. In 10 of these eperiments, an increase in clearance of 8 to 47 per cent occurred before, during, or after maximum hyperthermia. In five experiments there was no significant increase in the clearance. In only two of the 15 experiments did a decrease in clearance occur.

An increase in body temperature between 1.0° and 0.5° F. occurred during the exposure of a dog to a current of cold air at 38° and 47° F. in two experiments, during which the clearance increased 30 and 18 per cent, respectively.

Heating by the two electrical methods caused a reduction in the rate of urine flow irrespective of the region of heat production. On the other hand, the hyperthermias of typhoid vaccine injection and exposure to cold had no appreciable effect on the urine flow. A decrease in urine flow was due to an increase in the rate of tubular reabsorption of water and was independent of changes in the creatinine clearance.

REFERENCES

1. Goldring, W.: Observations With the Urea Clearance Test in Acute Rheumatic Infection, J. Clin. Investigation **10**: 345, 1931.
2. Grant, W. H., and Medes, G.: Creatinine Clearance During the Hyperthermia of Diathermy and Fevers, J. Lab. & Clin. Med. **20**: 345, 1934.

3. Page, I. H.: Effect of Diathermy Treatment of Kidneys on the Renal Function, J. A. M. A. **102**: 1131, 1934.
4. Farr, L. E., and Moen, J. K.: The Effect of Induced Hyperpyrexia on the Urea Clearance of Rheumatic Patients, Am. J. M. Sc. **197**: 53, 1939.
5. Merriman, J. R., Holmquest, H. J., and Osborne, S. L.: A New Method of Producing Heat in Tissues: The Inductotherm, Am. J. M. Sc. **187**: 667, 1934.
6. Mortimer, B., and Osborne, S. L.: Tissue Heating by Short Wave Diathermy, J. A. M. A. **104**: 1413, 1935.
7. Karr, J. W., and Nassett, E. S.: Physiological Effects of High Frequency Current, Am. J. Physiol. **107**: 170, 1934.
8. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W.: The Control of Renal Blood Flow and Glomerular Filtration in Normal Man, J. Clin. Investigation **17**: 683, 1938.
9. Smith, H. W.: Physiology of the Renal Circulation, The Harvey Lectures **35**: 166, 1939.
10. Miller, B. F., and Van Slyke, D. D.: A Direct Micro-titration Method for Blood Sugar, J. Biol. Chem. **114**: 583, 1936.
11. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. II.
12. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, J. Biol. Chem. **115**: 63, 1936.
13. White, H. L., and Heinbecker, P.: Interpretation of Diodrast Clearances in the Dog, Proc. Soc. Exper. Biol. & Med. **43**: 8, 1940.
14. White, H. L., and Rolf, D.: A Rapid Micro Method for Determining Diodrast and Inorganic Iodide Iodine in Blood and Urine, Proc. Soc. Exper. Biol. & Med. **43**: 1, 1940.

LABORATORY METHODS

GENERAL

A STUDY OF THE PHENOMENON OF ERYTHROCYTE SEDIMENTATION*

I. A Critical Survey of Literature

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IN ANY sedimenting system there are two components which must be considered—the suspending medium (dispersing phase) and that which is suspended (the dispersed phase). Blood within the vessels may well be said to have the components necessary to classify it as a system capable of presenting the phenomenon herein referred to as sedimentation. It has a fluid suspending medium (the plasma) and suspended material (the formed elements). A certain degree of sedimentation can be demonstrated even within the vessels themselves if samples at various levels between two tourniquets are examined.[73] Before samples of blood drawn from apparently healthy horses can clot, considerable separation of cells and plasma occurs, much as is reported in samples from human beings during severe disease and from women during the latter stages of normal pregnancy.[73]

However, to measure the complete phenomenon of sedimentation of the formed elements of blood in their own plasma in vitro necessitates even in the horse, with its naturally very rapid rate of sedimentation, the use of artificial means of preventing clotting. The effects of this necessary alteration in the normal status of the blood upon the ultimate measurement of the phenomenon of sedimentation in vitro will be discussed later. Suffice to say that one should recognize that the determination of sedimentation in vitro represents not the phenomenon as it might occur in unaltered blood but rather as it occurs in blood necessarily changed to prevent clotting.

Despite recognition of the fact that a complete theoretical explanation of the phenomenon of sedimentation is probably impossible due to its marked complexity, mention of some of the various factors thought to be involved and the consensus of some of the experimenters concerning the relative value of these factors is included herein principally to convey the source of my conception of the sedimentation phenomenon. Due cognizance is given the possibility that the opinions of some of these workers may have been acquired from results of techniques which have not measured the phenomenon completely.

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Reported data and opinions were segregated into six general groups: Physical Factors, Chemical Factors, Constancy, Physiological Factors, Technical Factors, and Pathological Factors. These were further subdivided where necessary. A conservative summary follows:

I. PHYSICAL FACTORS

a. *Cells vs. Plasma.*—Both cells and plasma are apparently necessary elements in sedimentation. Changes in any of the physical and chemical properties of cells or of plasma may exert a variable influence upon the rate of sedimentation, the magnitude of this effect apparently being dependent upon not only the degree of alteration in any of these properties but also the properties altered.[28, 48, 58, 62, 72, 73, 87, 96, 110, 111, 115, 138, 188, 213, 214, 217, 221, 227, 248, 253, 265, 321]

b. *Specific Gravity.*—It would seem reasonable that for settling to take place the specific gravity of the formed elements must be greater than the specific gravity of the plasma. However, it also seems reasonable that the *degree of change* in the ratio of specific gravity of formed elements and plasma cannot by itself explain the magnitude of alterations in the sedimentation velocity which are encountered.[24, 58, 72, 73, 115, 121, 149, 201, 214, 219, 225, 231, 301]

c. *Viscosity.*—It may be said that ever-present viscosity of the plasma resists the settling of formed elements in plasma but that *changes* in the sedimentation rate cannot be explained wholly upon the basis of *changes* in viscosity.[8, 17, 24, 25, 58, 67, 72, 73, 93, 115, 124, 130, 150, 187, 214, 225, 229, 231, 253, 255, 258, 263, 265, 301]

d. *Movement of Plasma.*—The general movement of plasma is upward and tends to retard settling.[72, 115, 150, 191, 241]

e. *Particle Size.*—It would seem that the size of the individual cell has an important bearing upon its fall, particularly in suspensions where there is no rouleaux formation or agglutination. In suspensions where cells aggregate, variations in the size of the agglomerates of cells bear a definite relationship to the changes in sedimentation of the agglomerates—the larger the agglomerate the more rapid its fall.[2, 22, 24, 31, 38, 40, 50, 52, 58, 72, 73, 115, 145, 150, 214, 220, 225, 227, 241, 242, 259, 263, 288, 301, 321, 344]

f. *Number of Particles.*—Variations in the number of particles in a suspension produce variations in their rate of fall—the more present the slower each individual particle will fall. Since the number of cells present may influence, among other things, the number of aggregates, there probably exists a relationship between the number of aggregates and their rate of fall; with blood as a sedimenting system the more aggregates the slower their rate of fall.[5, 18, 23-26, 29, 30, 33, 39, 48, 50-52, 58, 61, 67, 68, 72, 73, 76, 82, 89, 93, 96, 97, 99-102, 105-107, 109, 110, 113, 115, 125, 128, 136, 138, 142, 145, 150, 160, 170, 186, 190, 195, 203, 204, 211, 225, 227, 232, 235, 241, 242, 255, 257-259, 263-265, 272, 283, 288, 289, 318, 321, 322, 329-331, 333-335, 340, 341, 343, 344]

g. *Rouleaux Formation and Agglutination.*—It seems universally accepted that the rate of sedimentation is considerably dependent upon rouleaux formation and subsequent agglutination—the larger the agglomerates of cells the more rapid their rate of fall.[4, 17, 24, 28, 48, 58, 62, 63, 65, 72, 73, 82, 87, 93, 96, 115, 120, 125, 132, 141, 149, 150, 184, 188, 190, 194, 201, 214, 217, 221, 226, 227, 231, 236, 238, 241, 242, 253, 256, 258, 259, 263, 274, 280, 288, 289, 310, 321, 335, 338, 344, 348]

h. Environmental Temperature.—It seems commonly agreed that decreases in the temperature of samples are accompanied by decreases in the rate of sedimentation, and that increases in temperature are associated with increases in the rate. It would appear that there is some difference of opinion as to the effects of heating plasma.[8, 12, 61, 64, 72, 73, 79, 82, 93, 94, 96, 115, 145, 150, 156, 160, 164, 208, 211, 225, 227, 234, 241-243, 255, 258, 259, 283, 288, 300, 314, 321, 322, 329, 330, 340]

i. *Other Physical Forces.*—Apparently such forces as the electrical charge of the cell, potential difference, cohesive forces, surface forces, repulsive forces, and osmotic pressure, exert influence either directly upon the sedimentation of agglomerates themselves or indirectly upon the phenomena of rouleaux formation and agglutination.[5, 8, 24, 28, 41, 48, 70-73, 82, 87, 96, 129, 130, 132, 141, 149, 152, 157, 176, 179, 180, 182-184, 201, 203, 211, 217, 221, 234, 238, 242, 253, 258, 264, 265, 280, 285, 288, 312, 325, 326, 344]

II. CHEMICAL FACTORS

a. *Hemoglobin.*—That hemoglobin within normal ranges bears a fairly constant relationship to normal values of sedimentation is to be expected but correlation between *changes* in hemoglobin and *changes* in sedimentation is debatable.[5, 22, 31, 39, 40, 45, 48, 50, 99, 100, 106, 111, 115, 138, 203, 232, 259, 263, 264, 288, 321, 329, 330, 340, 344]

b. *Venous, Arterial, and Capillary Blood.*—The consensus seems to be that results from tests on venous, arterial, and capillary blood, as commonly obtained, agree.[28, 54, 164, 204, 245, 258, 288]

c. *Oxygen and Carbon Dioxide.*—While large variations in the oxygen or carbon dioxide content of blood experimentally may produce some alteration in sedimentation, simple aeration of blood prior to determination of sedimentation seems to be of no important significance.[26, 73, 82, 100, 139, 155, 172, 204, 211, 234, 235, 245, 258, 260, 261, 327]

d. *pH.*—There seems to be a difference of opinion as to whether variations in pH influence sedimentation even though some agreement exists that acidosis slows the rate and alkalosis hastens it.[3, 5, 7, 8, 87, 156, 174, 201, 207, 228, 232, 234, 274, 344]

e. *Protein Fractions.*—It would appear that increases in fibrinogen, and to a lesser extent perhaps globulin, have an accelerating effect upon sedimentation while increases in albumin have a retarding influence.[1, 5, 8, 9, 12, 23, 24, 26, 28, 37, 43, 46, 48, 50, 51, 53, 57-59, 69-73, 78, 80, 82, 84, 87, 89, 93, 96, 97, 104-107, 115, 122, 128, 130, 132, 135, 138, 142, 145, 147, 152, 160, 163, 170, 173, 179, 180, 188-190, 196, 199, 201, 204, 206, 213, 214, 218, 227, 230-234, 236, 245, 246, 253, 254, 257, 258, 263, 265, 267, 268, 271, 273-275, 277, 282, 288, 290-292, 299, 305, 312, 325, 327, 328, 330, 333, 337, 342-344, 348]

f. *Cholesterol and Lecithin.*—Increases in cholesterol are said to be accompanied by increases in sedimentation while increases in lecithin are said to be associated with decreases in sedimentation.[44, 51, 58, 112, 115, 161, 162, 213, 232, 241, 247, 253, 254, 263, 274, 299, 320, 325]

g. *Other Chemical Factors.*—That other chemical factors such as bile products, chlorides, lactic acid, lipoid equilibrium and nitrogen balance, may influence sedimentation is not doubted, but whether any of these can offer a complete explanation of the phenomenon of sedimentation is assumably dubious.[5, 8, 12, 25, 28, 39, 58, 96, 115, 130, 169, 182, 183, 234, 253, 254, 263, 265, 325, 344]

III. CONSTANCY

In the opinion of the majority a certain constancy of results during health apparently can be expected. However, there is enough opinion to the contrary to sug-

gest a careful critique of these techniques since a multiplicity of techniques have been employed.[15, 33, 53, 64, 103, 115, 134, 145, 164, 190, 211, 232, 235, 274, 286, 288, 312, 321, 343, 344]

The more literature that is reviewed the more one comes to realize that the phenomenon of sedimentation is very complex, and its measurement probably does not represent the measurement of any particular constituent or property or any limited group of constituents or properties of the blood sample being considered. It represents a measurement of a balance between, *on the one side*, the influence of *all* the constituents and properties, intrinsic or extrinsic, which augment the separation of the fluid and formed elements of a particular blood sample, altered so as to allow this separation to take place, and, *on the other side*, the influences of *all* the constituents and properties which delay this separation. If this conception be reasonable, then all constituents and properties, intrinsic and extrinsic, must be causal, even though variations in some may be of more relative importance than variations in others.

It would appear that alterations in the balance of these opposing constituents and properties may be the result of changes in the body itself (intrinsic) *and* changes brought about by the necessary manipulation of the blood sample (extrinsic or technical). Since the clinical value of the test probably lies in its use as a delicate measure of the intrinsic changes in the particular sample the result of disease, then the intrinsic changes of a normal physiologic nature and the extrinsic or technical factors which may produce changes in the manifestation of the phenomenon must be enumerated, evaluated, controlled or allowed for before the measurement of sedimentation can be said to reflect pathology.

IV. PHYSIOLOGICAL FACTORS

a. *Sex.*—The majority believe that the rate of sedimentation is faster in adult women than in adult men. There is some difference of opinion as to whether this difference can be accounted for solely by the difference in volume per cent of cells as noted between men and women.[10, 53, 72, 73, 96, 103, 105, 106, 108, 137, 138, 202, 259, 264, 340, 343]

b. *Pregnancy.*—Pregnancy is recognized as causing an increase in sedimentation, being first noted after the first few months, reaching its peak at parturition and gradually returning toward normal during puerperium.[8, 11, 14, 27, 28, 48, 51, 57, 71-74, 82, 93, 96, 103, 127, 138, 180, 183, 184, 194, 201, 202, 211, 233, 259, 267, 270, 274, 285, 322, 330, 343]

c. *Menstruation.*—Some authors find an increase in sedimentation during menstruation. Others doubt an increase above normal limits.[53, 103, 138, 180, 181, 274, 295, 322, 340, 343]

d. *Age.*—A difference of opinion exists regarding age but the majority find a slower sedimentation in adults than in young or old persons.[53, 72, 73, 105, 138, 198, 202, 211, 225, 288, 301, 312, 343, 346]

e. *Food.*—That ingestion of food has no effect upon sedimentation seems at least generally accepted.[48, 61, 97, 105, 145, 211, 258]

f. *Exercise.*—Again a difference of opinion exists. Probably the degree of exercise is important since enforced rest is reported as delaying sedimentation and overwork as increasing it.[211, 225, 232, 258]

g. *Sweating.*—Profuse sweating by electric baking had no effect on sedimentation.[8]

h. *Excitement.*—Tests taken at times of greater excitement in children gave higher readings.[312]

i. *Time of Day.*—A difference of opinion exists as to whether or not the time of day has any direct effect upon the sedimentation rate.[134, 164, 211, 258]

j. *Atmospheric Conditions.*—Atmospheric conditions are reported as having some effect on sedimentation.[94, 134, 146, 225, 301, 312]

V. TECHNICAL FACTORS

1. It would seem that *agreeable results* upon the same sample can be obtained with certain techniques. However, literature upon this subject is very deficient and techniques differ considerably.[72, 115, 308, 340]

2. It seems commonly accepted that *venous stasis* should at least be minimized, since if it is applied in excess, sedimentation is usually considerably retarded.[82, 93, 145, 204, 211, 232, 234, 259, 264, 265, 288, 321, 330]

3. Too little *anticoagulant*, as well as too much anticoagulant, is to be avoided. Anticoagulant and blood must be properly mixed. Different anticoagulants yield different results. Although solutions of sodium citrate have probably been used more than any other anticoagulant, the present tendency is to lean toward the anticoagulant mixture of *Heller and Paul*[123] and toward heparin, for these two seemingly produce little variation in cell volume as well as sedimentation. Differences of opinion naturally exist concerning the proper kind and amount of anticoagulant to use.[8, 20, 23, 29, 32, 39, 50, 51, 58, 60, 64, 72, 76, 95, 101, 105, 109, 115, 119, 123, 125, 133, 149, 180, 216, 221, 224, 232, 235, 241, 245, 249, 257, 258, 264, 280, 288, 311, 321, 322, 329, 340, 341, 345]

4. It would seem that the general consensus is that *delay* in conducting a measurement of the sedimentation of a sample may cause retardation of the rate. The maximum amount of permissible delay reported is variable from a few hours to several hours. It is suggested that the retarding effect of delay may bear some relationship to the kind of anticoagulant used and also the temperature at which the sample is kept.[29, 51, 54, 60, 64, 72, 75, 93, 108, 115, 119, 136, 142, 154, 156, 164, 185, 186, 201, 211, 224, 227, 239, 240, 242, 258, 259, 281, 283, 288, 329, 330, 338, 340, 344, 348]

5. Although it has been recognized that a *uniform mixture of cells and plasma* is necessary at the start of measurement of sedimentation, it would seem that little attempt has been made to demonstrate that such a uniformity is actually established.[50, 55, 94, 115, 136, 154, 164, 185, 211, 258, 259, 308]

6. There has been a multiplicity of *tubes* used for the measurement of sedimentation. However, there seem to be certain generalities which are quite commonly accepted. Capillary tubes are thought not to be dependable. The majority of workers used tubes of over 2 mm. bore and apparently thought them satisfactory. The general opinion seems to be that longer tubes yield faster rates, yet there is reason to doubt this upon a physical basis. It seems recognized that the sides of tubes should be parallel and the bottoms should be flat.[8, 10, 12, 17-19, 26, 28, 29, 35, 36, 50, 51, 54, 55, 57, 58, 60, 64, 72, 76, 78, 79, 82, 94, 96, 101, 106, 108, 109, 114, 115, 118, 137, 145, 154, 164, 171, 180, 193, 198, 204, 211, 214, 225, 227, 234, 235, 241, 250, 258, 259, 263-265, 273, 278, 287, 288, 293, 315, 317, 321, 322, 325, 329, 330, 340, 341, 343, 348]

7. The necessity of *perpendicularity of the tube* seems generally recognized.[12, 17, 19, 29, 34, 36, 42, 64, 73, 79, 114, 115, 154, 164, 191, 193, 210, 211, 225, 227, 235, 241, 259, 264, 293, 317, 321, 330, 340, 348]

8. It appears generally accepted that sedimentation takes place in three *stages:* (a) Initial aggregation and accelerated fall. (b) Constant fall. (c) Slowing or retarded fall.

There is evidence that sedimentation occurs as a sigmoid curve. This would render dubious the possibility of a phase of constant fall.[17, 29, 33, 58, 64, 73, 115, 191, 215, 259]

9. There exists a great variety of *methods of expressing sedimentation,* the great majority of which depend upon a relationship between the position of the line of demarcation between cells and plasma and the amount of time since the test was begun. Some have used the amount of time necessary for the border between cells and plasma to reach a certain mark, some have noted the position of this line at a given time, and others have expressed the descent of this border line by reading its position at definite time intervals. Many of the mentioned methods probably fail to express the phenomenon *completely* and some probably do not represent expression of *any part* of what is generally considered the most important phase of the phenomenon, namely, aggregation. Multiple readings of the degree of settling at frequent time intervals or mechanical means of measuring the rate seem to offer advantages.

It is felt that evidence from results with an automatic photographic device, which continuously records the rate of descent of the line of demarcation between cells and plasma,[215] is convincing that the best measurement of the phenomenon of sedimentation, as evidenced by the rate of descent of the line of demarcation between cells and plasma, is a continuous measurement with estimation of the point of change in the resulting sigmoid curve. Such estimation, if even partially to express the important aspects of the curve, must include the amount of separation before the point of change takes place, the time at which the point of change occurs, and the rate or velocity of separation immediately preceding the point of change (expressed as an angle between the steepest portion of the sigmoid curve and perpendicularity).[7, 10, 12, 17-19, 23-25, 29, 30, 33, 36, 37, 50, 53, 55, 57-59, 61, 64, 68, 69, 72, 73, 76, 79, 88, 93, 94, 96, 99-101, 103, 105-108, 110, 113-115, 118, 119, 134, 136, 137, 150, 154, 159, 164, 171, 180, 181, 186, 190, 194, 195, 198, 201, 211, 214, 215, 218, 225, 227, 235, 241, 258, 259, 264, 265, 273, 278, 287-289, 293, 295, 298, 301, 302, 308, 315, 317, 321, 325, 328-331, 333, 334, 340]

VI. PATHOLOGICAL FACTORS

Inclusion of several references brings out the generally accepted theory that alteration of the sedimentation phenomenon portrays metabolic changes—the result of nonspecific destructive disease.[5, 11, 28, 33, 47, 48, 51, 53, 55, 57, 72, 76, 78, 79, 87, 103, 110, 114, 115, 174, 195, 198, 201, 211, 259, 265, 274, 325, 330, 339, 343]

The second of this series of articles will deal with the establishment of a reliable technique with evidence of its reproducibility and limitations.

REFERENCES

1. Abderhalden: München. med. Wchnschr. **68:** 973, 1921.
2. Idem: Pflüger's Arch. f. d. ges. Physiol. **193:** 263, 1922.
3. Abramson: J. Gen. Physiol. **14:** 163, 1930.
4. Idem: Electrokinetic Phenomena and Their Application to Biology and Medicine, The Chemical Catalogue Company, Inc., 1934, New York City.
5. Achard and Co-workers: Arch. d. mal. du coeur. **24:** 657, 1931. Abst. J. A. M. A. **98:** 854, 1932.
6. Adams-Ray: Acta chir. Scandinav. **66:** 263, 1930.

7. Aggazzotti and Co-workers: Boll. Soc. ital. biol. sper. **6**: 159, 1931.
8. Alexander: Med. J. & Rec. **119**: 549, 1924.
9. Andrel: Essai d. hematologie pathologique, Paris, 1845.
10. Answer in editorial: J. A. M. A. **91**: 1915, 1928.
11. Ibid: J. A. M. A. **92**: 413, 1929.
12. Bach and Hill: Lancet, No. 5654, p. 75, 1932.
13. Baer and Reis: Tr. Am. Gynec. Soc. **50**: 291, 1925.
14. Idem: Surg., Gynec. & Obst. **40**: 691, 1925.
15. Idem: Am. J. Obst. & Gynec. **10**: 397, 1925.
16. Balachowsky: Presse méd. **33**: 629, 1925.
17. Idem: Ann. de méd. **18**: 201, 1925.
18. Bannick and Co-workers: J. A. M. A. **109**: 1257, 1937.
19. Banyai and Anderson: Arch. Int. Med. **46**: 787, 1930.
20. Barrat: J. Physiol. **90**: 377, 1937.
21. Barth: München. med. Wchnschr. **83**: 202, 1938.
22. Behrens: München. med. Wchnschr. **71**: 229, 1924.
23. Bendien and Snapper: Biochem. Ztschr. **235**: 14, 1931.
24. Bendien and Co-workers: Biochem. Ztschr. **247**: 306, 1932.
25. Bertrand and Rousseaux: Rev. franç. d'endocrinol. **9**: 445, 1931.
26. Biernacki and Muller: Wien. klin. Wchnschr. **19**: 539, 1906.
27. Bland: Surg., Gynec. & Obst. **50**: 429, 1930.
28. Bochner and Wassing: J. Lab. & Clin. Med. **11**: 214, 1935.
29. Boerner and Flippin: J. Lab. & Clin. Med. **20**: 583, 1935.
30. Bonniger and Herrmann: Klin. Wchnschr. **2**: 744, 1923.
31. Idem: Klin. Wchnschr. **3**: 403, 1924.
32. Bonniger: Deutsche med. Wchnschr. **53**: 269, 1927.
33. Bouton: J. Lab. & Clin. Med. **23**: 519, 1938.
34. Boycott: Nature **104**: 532, 1920.
35. Branch and Co-workers: Chinese M. J. **40**: 27, 1926.
36. Brooks: J. Lab. & Clin. Med. **21**: 971, 1936.
37. Broom: J. Lab. & Clin. Med. **22**: 998, 1937.
38. Bunting: Am. J. M. Sc. **198**: 191, 1939.
39. Burke and Weir: J. Lab. & Clin. Med. **18**: 657, 1933.
40. Burker: München. med. Wchnschr. **69**: 577, 1922.
41. Burkzeller and Stanker: Internat. Ztschr. f. phys.-chem. Biol. **3**: 133, 1917.
42. Burkzeller and Wastl: Biochem. Ztschr. **142**: 524, 1923.
43. Buscher: Berl. klin. Wchnschr. No. 14, 1921. Quoted by Reyner: J. Lab. & Clin. Med. **14**: 630, 1929.
44. Byrom and Kay: Brit. J. Exper. Path. **9**: 72, 1928.
45. Castex and Schteingart: Rev. Soc. argent. de biol. **7**: 491, 1931.
46. Chandler: J. Lab. & Clin. Med. **12**: 1092, 1927.
47. Cherry: Am. J. Obst. & Gynec. **11**: 105, 1926.
48. Idem: J. Lab. & Clin. Med. **20**: 257, 1934.
49. Chung: Chinese M. J. **48**: 1101, 1934.
50. Idem: J. Lab. & Clin. Med. **20**: 633, 1935.
51. Cooper: J. Lab. & Clin. Med. **11**: 615, 1926.
52. Cunningham: Proc. Roy. Soc., Sec. A. **83**: 357, 1909-10.
53. Cutler: Am. J. M. Sc. **171**: 882, 1926.
54. Idem: Am. J. M. Sc. **173**: 687, 1927.
55. Idem: Am. Rev. Tuberc. **19**: 544, 1929.
56. Cutler and Cohen: Am. Rev. Tuberc. **21**: 347, 1930.
57. Cutler: Am. J. M. Sc. **183**: 643, 1932.
58. Cutler and Associates: Am. J. M. Sc. **195**: 734, 1938.
59. Davis: J. Lab. & Clin. Med. **21**: 478, 1936.
60. Dawson and Co-workers: J. Lab. & Clin. Med. **15**: 1065, 1930.
61. De Courcy: Am. J. Surg. **39**: 129, 1925.
62. De Hann: Biochem. Ztschr. **86**: 298, 1918.
63. Dogiel: Arch. f. Anat. u. Physiol. pp. 357-364, 1885.
64. Dorfman and Brooks: J. Lab. & Clin. Med. **22**: 510, 1937.
65. Dyke: Lancet **1**: 579, 1922.
66. Editorial: North Am. Vet., September, p. 24, 1938.
67. Einstein: Ann. de Physik **19**: 289, 1906; Ibid. **34**: 590, 1911; Kolloid-Ztschr. **27**: 137, 1920.
68. Engel: Wien. Arch. f. inn. Med. **9**: 45, 1924.
69. Ernstene: Am. J. M. Sc. **180**: 12, 1930.
70. Fahraeus: Hygeia **80**: 369, 1918.
71. Fahraeus: Biochem. Ztschr. **89**: 115, 1918.
72. Idem: Acta med. Scandinav. **55**: 1, 1921.
73. Idem: Physiol. Rev. **9**: 241, 1929.
74. Falta: Zentralbl. f. Gynäk. **48**: 1478, 1924.

75. Feldman: Am. J. M. Sc. **200:** 820, 1940.
76. Ferguson: J. Am. Vet. M. A. **91:** 163, 1927.
77. Fetter and Schnabel: Am. J. M. Sc. **201:** 115, 1941.
78. Fischel: Am. Rev. Tuberc. **10:** 606, 1925.
79. Forman: J. Lab. & Clin. Med. **12:** 373, 1927.
80. Foster: Arch. Int. Med. **34:** 301, 1924.
81. Freudenthal: Ugesk. f. laeger. **95:** 957, 1933.
82. Friedlaender: Am. J. Obst. & Gynec. **7:** 125, 1924.
83. Idem: Ugesk. f. laeger. **95:** 896, 1933.
84. Frisch and Starlinger: Med. Klin. **17:** 1147, 1921.
85. Idem: Med. Klin. **18:** 247, 1922.
86. Frisch: Beitr. z. Klin. d. Tuberk. **60:** 141, 1924.
87. Frosch: J. Lab. & Clin. Med. **11:** 43, 1925.
88. Gallagher: Am. J. M. Sc. **188:** 450, 1934.
89. Gibson and Banzhof: J. Exper. Med. **12:** 411, 1910.
90. Gibson: Proc. Roy. Soc. Med. **31:** 309, 1938.
91. Gilbert and Associates: Compt. rend. Soc. de biol. **88:** 873, 1923.
92. Idem: Compt. rend. Soc. de biol. **94:** 837, 1926.
93. Gilligan and Ernstene: Am. J. M. Sc. **187:** 552, 1934.
94. Gordon and Cohn: Am. J. M. Sc. **176:** 211, 1928.
95. Gough: Biochem. J. **18:** 202, 1924.
96. Gram: Arch. Int. Med. **28:** 312, 1921.
97. Idem: Ibid. **56:** 107, 1922.
98. Gram and Norgaard: Arch. Int. Med. **31:** 164, 1923.
99. Gram: Acta med. Scandinav. **68:** 108, 1928.
100. Idem: Ibid. **70:** 242, 1929.
101. Gregg: J. Lab. & Clin. Med. **22:** 786, 1937.
102. Greisheimer: Am. Rev. Tuberc. **16:** 344, 1927.
103. Idem: Am. J. M. Sc. **174:** 338, 1927.
104. Idem: Am. J. Dis. Child. **37:** 953, 1929.
105. Greisheimer and associates: Am. J. M. Sc. **177:** 816, 1929.
106. Idem: Am. J. Physiol. **89:** 170, 1929.
107. Idem: Am. Rev. Tuberc. **19:** 559, 1929.
108. Idem: Am. J. M. Sc. **187:** 213, 1934.
109. Idem: Am. J. M. Sc. **190:** 775, 1935.
110. Griffin and Brose: J. Am. Vet. M. A. **89:** 664, 1936.
111. Groedel and Hubert: Ztschr. f. klin. Med. **102:** 31, 1925.
112. Grossmann: Ztschr. f. d. ges. exper. Med. **42:** 496, 1925.
113. Guthmann and Schneider: Arch. f. Gynäk. **127:** 515, 1926.
114. Halpenny: Irish J. M. Sc. Jan. p. 30, 1932. Quoted in abstract, Am. J. Dis. Child. **45:** 1093, 1933.
115. Ham and Curtus: Medicine **17:** 447, 1938.
116. Hambleton and Christianson: Am. J. M. Sc. **198:** 177, 1939.
117. Hammersland and Associates: J. Am. Vet. M. A. **93:** 320, 1938.
118. Hardt: Maandsckr. Dyrl. **45:** 171, 1933.
119. Haskins and Co-workers: J. Lab. & Clin. Med. **16:** 487, 1931.
120. Hayem: Du Sang et ses alterations anatomiques, Paris, 1889, p. 473.
121. Heilmeyer: Handbook of General Hematology **2:** 329, 1933.
122. Hekma: Biochem. Ztschr. **7:** 177, 1908.
123. Heller and Paul: J. Lab. & Clin. Med. **19:** 777, 1934.
124. Herzfeld and Schinz: Strahlentherapie **15:** 84, 1923.
125. Hesser: Acta. med. Scandinav. **61:** 1, 1924.
126. Hewson: Works edited by Gulliver, London, Vol. 1, 1846.
127. Hirst and Long: Am. J. M. Sc. **171:** 846, 1926.
128. Hiss and Atkinson: J. Exper. Med. **5:** 47, 1900.
129. Hober: Arch. f. d. ges. Physiol. **101:** 627, 1904.
130. Idem: München. med. Wchnschr. **69:** 1617, 1922.
131. Hodgman: Handbook of Chemistry and Physics, ed. 22, Cleveland, 1937, Chemical Rubber Publishing Co.
132. Hoeber and Mond: Klin. Wchnschr. **1:** 2412, 1922.
133. Hooper and Associates: Am. J. Physiol. **51:** 205, 1920.
134. Hoverson and Petersen: Am. J. M. Sc. **188:** 455, 1934.
135. Howe: Physiol. Rev. **5:** 439, 1925.
136. Hubbard and Geiger: J. Lab. & Clin. Med. **12:** 322, 1928.
137. Hunt: J. Lab. & Clin. Med. **13:** 327, 1928.
138. Idem: Ibid. **14:** 1061, 1929.
139. Ito: Tohoku J. Exper. Med. **5:** 139, 1924.
140. Jaques: Haver-Glover Messenger, Jan.-Feb., 1941, p. 40.
141. Jevons: Tr. Manchester Phil. Soc. No. 78, 1870.
142. Joachin: Pflüger's Arch. f. d. ges. Physiol. **93:** 558, 1902.

143. Joltrain and Associates: Rev. de méd., Paris **46**: 543, 1929.
144. Jones: U. S. Vet. Bur. M. Bull. **1**: 5, 1926.
145. Idem: J. Lab. & Clin. Med. **15**: 209, 1930.
146. Kapp: Beitr. z. Klin. d. Tuberk. **68**: 378, 1928.
147. Katz: Ztschr. f. Tuberk. **35**: 401, 1922.
148. Katz and Radt: Med. Klin. **23**: 760, 1927.
149. Kegerreis: J. Lab. & Clin. Med. **22**: 581, 1937.
150. Kermack and Associates: Proc. Roy. Soc. Edinburgh **49**: 170, 1929.
151. Kilduffe: J. Lab. & Clin. Med. **15**: 54, 1930.
152. Kimura and Nakazawa: Physiol. Abst. **17**: 180, 1932.
153. Klinch: Am. J. M. Sc. **181**: 743, 1931.
154. Kling: Arch. Int. Med. **50**: 419, 1932.
155. Koehler and Associates: J. Biol. Chem. **64**: 313, 1925.
156. Koster: Acta med. Scandinav. **93**: 420, 1937.
157. Kozawa: Biochem. Ztschr. **60**: 146, 1914.
158. Kral: 12th Internat. Vet. Cong. Paper 19, 1934.
159. Kramer: J. Lab. & Clin. Med. **21**: 37, 1935.
160. Krautz: Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**: 507, 1937.
161. Kurten: Arch. f. d. ges. Physiol. **185**: 248, 1920.
162. Idem: Klin. Wchnschr. **3**: 1216, 1924.
163. Kylin: Acta med. Scandinav. **85**: 574, 1935.
164. Landau: Am. J. Dis. Child. **45**: 691, 1933.
165. Langer and Schmidt: Ugesk. f. laeger. **95**: 896, 1933.
166. Lasch: Ztschr. f. d. ges. exper. Med. **42**: 548, 1925.
167. Lebel and Lottrup: Hospitalstid. **76**: 597, 1933.
168. Idem: Acta med. Scandinav. **81**: 239, 1934.
169. Lederer: Monatschr. f. Kinderh. **27**: 608, 1924.
170. Ledingham: J. Hyg. **7**: 65, 1907.
171. Lee: Am. J. M. Sc. **195**: 729, 1938.
172. Leendertz: Deutsches Arch. f. klin. Med. **137**: 234, 1921.
173. Levinson: Am. Rev. Tuberc. **7**: 264, 1923.
174. Ley: Arch. f. d. ges. Physiol. **197**: 399, 1922.
175. Liege and Co-workers: Bull. Soc. de pédiat. de Paris **30**: 93, 1932.
176. Liffkowitz: Ergebn. d. ges Med. **17**: 171, 1932.
177. Lingren: Acta med. Scandinav. **93**: 590, 1937.
178. Lintz: J. Lab. & Clin. Med. **21**: 1259, 1936.
179. Linzinmeier: Pflüger's Arch. f. d. ges. Physiol. **185**: 176, 1920.
180. Idem: Arch. f. Gynäk. **113**: 608, 1920.
181. Idem: Zentralbl. f. Gynäk. **44**: 816, 1920.
182. Idem: Pflüger's Arch. f. d. ges. Physiol. **186**: 272, 1921.
183. Idem: München. med. Wchnschr. **70**: 1243, 1923.
184. Idem: Ztschr. f. arztl. Fortbild. **20**: 445, 1923.
185. Idem and Raunert: Zentralbl. f. Gynäk. **48**: 786, 1924.
186. Logie: J. Florida M. A. **24**: 467, 1938.
187. Lohr: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **34**: 229, 1922.
188. Lucie and Associates: Proc. Soc. Exper. Biol. & Med. **32**: 189, 1934.
189. Idem: Ibid. **33**: 356, 1935.
190. Idem: Am. J. M. Sc. **192**: 179, 1936.
191. Lundgren: Acta med. Scandinav. **67**: 63, 1927.
192. Madlener and Associates: Beitr. z. klin. Chir. **149**: 425, 1930.
193. Maia: Compt. rend. Soc. de biol. **102**: 248, 1929.
194. Manzani: Arch. de physique biol. **9**: 97, 1931.
195. McGee: J. A. M. A. **110**: 718, 1938.
196. McLester: J. A. M. A. **79**: 17, 1922.
197. Meeker: Clifton M. Bull. **11**: 45, 1925.
198. Miller: J. Lab. & Clin. Med. **21**: 1227, 1936.
199. Moen and Associates: J. Clin. Investigation **12**: 589, 1933.
200. Molnar: Abst. Am. J. Obst. & Gynec. **7**: 501, 1924.
201. Monaghan and White: J. Gen. Physiol. **19**: 715, 1936.
202. Mora and Gault: J. Lab. & Clin. Med. **15**: 590, 1930.
203. Morriss: Am. Rev. Tuberc. **10**: 431, 1924.
204. Morriss and Rubin: J. Lab. & Clin. Med. **11**: 1045, 1926.
205. Muller: Beobach. über spont. Blut sedimentierung, Berlin, 1898.
206. Murakami and Yamaguchi: Ann. de méd. **15**: 297, 1924.
207. Nakamura and Kuroda: J. Biochem., Japan **25**: 555, 1937.
208. Idem: Keijo J. Med. **8**: 168, 1937.
209. Idem: Ibid. **8**: 198, 1937.
210. Idem: Ibid. **8**: 256, 1937.
211. Nees: U. S. Nav. M. Bull. **23**: 471, 1925.
212. Netter: Arch. f. d. ges. Physiol. **308**: 16, 1925.

213. Newham: Quart. J. Med. **20**: 371, 1926.
214. Newham and Martin: Quart. J. Med. **22**: 145, 1928.
215. Nichols: J. LAB. & CLIN. MED. **24**: 631, 1939.
216. Nolf: Medicine **17**: 381, 1938.
217. Northrup and Freund: J. Gen. Physiol. **6**: 603, 1923.
218. Oakley: Lancet **234**: 312, 1938.
219. Oestreich: Klin. Wchnschr. **10**: 160, 1931.
220. Ohno: Pflüger's Arch. f. d. ges. Physiol. **202**: 376, 1923.
221. Oliver and Barnard: J. Gen. Physiol. **7**: 99, 1924.
222. Olovsen: Hygeia **94**: 433, 1932.
223. Osgood and Baker: Arch. Int. Med. **37**: 685, 1926.
224. Osgood and Haskins: Laboratory Diagnosis, Philadelphia, 1931, P. Blakiston's Sons & Co.
225. Panisset: Canad. J. Comp. Med. **2**: 3, 1938.
226. Panum: Dissertation, Copenhagen, 1851.
227. Payne: Lancet No. 5654, p. 74, 1932.
228. Peters: J. Clin. Investigation **1**: 435, 1924-5.
229. Petschacher: Ztschr. f. Tuberk. **20**: 284, 1923.
230. Idem: Verhandl. d. deutsch. Gesellsch f. inn. Med. April, 1923. Quoted by Reyner: J. LAB. & CLIN. MED. **14**: 630, 1929.
231. Pfeiffer: Ztschr. f. klin. Med. **33**: 215, 1897.
232. Pinner and Co-workers: Arch. Path. **5**: 810, 1928.
233. Plass and Matthew: Am. J. Obst. & Gynec. **12**: 3, 1926.
234. Plass and Co-workers: J. LAB. & CLIN. MED. **12**: 735, 1926.
235. Plass and Rourke: J. Clin. Investigation **5**: 531, 1928.
236. Plaut: München. med. Wchnschr. **67**: 279, 1920.
237. Ploman: Ann. d'ocul. **157**: 1920. Quoted by Fahraeus: Physiol. Rev. **9**: 241, 1929.
238. Pohle: Radiology **5**: 206, 1925.
239. Idem: Ibid. **6**: 55, 1926.
240. Ponder: Quart. J. Exper. Physiol. **14**: 334, 1924.
241. Idem: Ibid. **15**: 236, 1925.
242. Idem: Ibid. **16**: 173, 1926.
243. Ponder: Quart. J. Exper. Physiol. **22**: 281, 1932.
244. Idem: Ibid. **23**: 291, 1933.
245. Popper and Kreindler: Ann. de méd. **17**: 57, 1925.
246. Idem: Paris méd. **61**: 129, 1926.
247. Pribram and Klein: Acta med. Scandinav. **58**: 132, 1923.
248. Puxeddu: Riforma med. **38**: 819, 1922.
249. Reed: J. LAB. & CLIN. MED. **14**: 243, 1928.
250. Reichel: Blutkorperchensenkung, Vienna, 1936, J. Springer.
251. Remen: Klin. Wchnschr. **10**: 2113, 1931.
252. Rest: Tubercle, London **19**: 289, 1938.
253. Reyner: J. LAB. & CLIN. MED. **14**: 630, 1929.
254. Rosenthal and Blowstein: J. LAB. & CLIN. MED. **14**: 464, 1929.
255. Rosenthal: Med. J. Australia **1**: 172, 1937.
256. Rothe: Deutsche med. Wchnschr. **50**: 44, 1924.
257. Rourke and Plass: Am. J. Physiol. **84**: 42, 1928.
258. Idem: J. Clin. Investigation **7**: 365, 1929.
259. Rourke and Ernstene: J. Clin. Investigation **8**: 545, 1930.
260. Roussy and Mosinger: Compt. rend. Soc. de biol. **126**: 1064, 1937.
261. Rowe: J. LAB. & CLIN. MED. **1**: 485, 1916.
262. Rubin: Surg., Gynec. & Obst. **42**: 652, 1926.
263. Idem: Arch. Int. Med. **37**: 848, 1926.
264. Rubin and Smith: Arch. Int. Med. **39**: 303, 1927.
265. Rubin: Am. J. M. Sc. **174**: 860, 1927.
266. Runnstrom and Schow: Acta Zool. **6**: 131, 1920.
267. Russell and Boyd: Am. J. Physiol. **99**: 424, 1932.
268. Sachs: Kolloid-Ztschr. **24**: 113, 1919.
269. Sachs and Oettinger: München. med. Wchnschr. **68**: 351, 1921.
270. Sakae and Tsutsumi: Japan M. World **1**: 1, 1921.
271. Salmon: Ztschr. f. klin. Med. **99**: 329, 1924.
272. Schafer: Arch. f. Gynäk. **130**: 566, 1927.
273. Schappes: Folia haemat. **58**: 160, 1937.
274. Schattenberg: Arch. Int. Med. **50**: 569, 1932.
275. Schindera: Deutsches Arch. f. klin. Med. **144**: 113, 1924.
276. Schloss and Miller: Am. J. M. Sc. **177**: 523, 1929.
277. Schmitz and Schmitz: Am. J. Obst. & Gynec. **11**: 353, 1926.
278. Schulhof: J. A. M. A. **100**: 318, 1933.
279. Schumaker and Vogel: Arch. f. Gynäk. **119**: 127, 1923.
280. Schwyzer: Biochem. Ztschr. **60**: 297, 1914.
281. Secker: J. Physiol. **60**: 286, 1926.

282. Seki: Biochem. Ztschr. **143:** 365, 1923.
283. Sellards: Bull. Johns Hopkins Hosp. **19:** 271, 1908.
284. Sharpless: Atlantic M. J. **31:** 10, 1927.
285. Schurer and Eimer: Berl. klin. Wchnschr. **63:** 1251, 1921.
286. Simms: J. Am. Vet. M. A. **96:** 77, 1940.
287. Smith and Associates: Am. J. M. Sc. **189:** 383, 1935.
288. Smith: Am. J. M. Sc. **192:** 73, 1936.
289. Smoluchowski: Ztschr. f. physiol. Chem. **92:** 129, 1917.
290. Starling: Principles of Human Physiology, London, 1920, p. 912.
291. Starlinger and Frosch: Med. Klin. **17:** 1147, 1921.
292. Starlinger: Deutsche med. Wchnschr. **54:** 731, 1928.
293. Steiger: Med. Klin. **29:** 1108, 1933.
294. Steinberg: Am. J. M. Sc. **190:** 98, 1935.
295. Stemmler: Arch. f. klin. Chir. **137:** 705, 1925.
296. Stern: Deutsches ges. f. kinderh. **9:** 1924. Quoted by Rubin: Arch. Int. Med. **37:** 848, 1926.
297. Stewart and Holman: Vet. Rec. **52:** 157, 1940.
298. Sukowitch: Am. J. M. Sc. **187:** 65, 1934.
299. Swedin: Biochem. Ztschr. **257:** 411, 1933.
300. T'ang: Chinese M. J. **51:** 445, 1937.
301. Tapernoux and Pagnon: Canad. Roy. Soc. Biol. **115:** 64, 1933.
302. Theorell and Widstrom: Ztschr. f. d. ges. exper. Med. **75:** 668, 1931.
303. Theorell: Biochem. Ztschr. **268:** 46, 1934.
304. Theorell and Thorling: Klin. Wchnschr. **16:** 963, 1937.
305. Tiffeneau and Gysin: Compt. rend. Soc. de biol. **126:** 1160, 1937.
306. Trail and Associates: Lancet **1:** 179, 1929.
307. Trail: Lancet No. 5715, p. 522, 1933.
308. Treloar and Greisheimer: Proc. Soc. Exper. Biol. & Med. **30:** 962, 1933.
309. Turner and Herman: Mo. Agr. Exp. Sta. Bull. No. 159, 1931.
310. Valentin: Pathologie die Blutes, Leipzig, 1866-1867.
311. Val Allen: J. Lab. & Clin. Med. **10:** 1027, 1925.
312. Van Antwerp: Am. J. Dis. Child. **48:** 814, 1934.
313. Van der Hulst: Arch. internat. de pharmacodyn. et de thérap. **57:** 129, 1937.
314. Venischek and Douglass: Am. J. Obst. & Gynec. **14:** 200, 1927.
315. Vickers and Duryee: J. Lab. & Clin. Med. **18:** 260, 1932.
316. Vignes: Abst. Am. J. Obst. & Gynec. **7:** 501, 1924.
317. Villela: J. Lab. & Clin. Med. **20:** 1082, 1935.
318. Vogel: Arch. f. Gynäk. **134:** 129, 1928.
319. Von Mocsy: 12th Internat. Vet. Cong. 1934, Vol. 2, paper 18.
320. Vorschutz: Arch. f. exper. Path. u. Pharmakol. **95:** 235, 1922.
321. Walton: J. Lab. & Clin. Med. **18:** 711, 1933.
322. Walton: Quart. J. Med. **26:** 79, 1933.
323. Warren: Clifton M. Bull. **12:** 73, 1926.
324. Waugh: Canad. M. A. J. **13:** 604, 1922.
325. Weiss: Am. J. M. Sc. **181:** 379, 1931.
326. Wells and associates: Chemistry of the Blood in Tuberculosis, Baltimore, 1923, Williams & Wilkins Co.
327. Westergren: Acta med. Scandinav. **54:** 247, 1921.
328. Idem: Brit. J. Tuberc. **15:** 72, 1921.
329. Idem: Ergebn. d. inn. Med. u. Kinderh. **26:** 577, 1924.
330. Idem: Am. Rev. Tuberc. **14:** 94, 1926.
331. Idem: Acta med. Scandinav. **68:** 108, 1928.
332. Idem: Intern. Clin. p. 70, March, 1928. (Referred to in Editorial, J. A. M. A. **92:** 413, 1929.
333. Idem: Ztschr. f. d. ges. exper. Med. **75:** 668, 1931.
334. Idem: Acta med. Scandinav. **77:** 469, 1932.
335. Westgren and Reitstotter: Ztschr. f. physiol. Chem. **92:** 750, 1918.
336. Whipple and Hurwitz: J. Exper. Med. **13:** 136, 1911.
337. Whipple: Am. J. Physiol. **33:** 50, 1914.
338. Wiltshire: J. Path. & Bact. **17:** 282, 1912.
339. Wintrobe: Am. J. M. Sc. **185:** 58, 1933.
340. Wintrobe and Landsberg: Am. J. M. Sc. **189:** 102, 1935.
341. Wirth: Grundlagen einer Klinischen Hamatologie der Haustiere, Berlin, Vienna, 1931, Urban & Schwarzenberg.
342. Zarday and Farkas: Ztschr. f. d. ges. exper. Med. **78:** 367, 1931.
343. Zeckwer and Goodell: Am. J. M. Sc. **169:** 209, 1935.
344. Zippel: Folia haemat. **43:** 486, 1931.
345. Gram and Norgaard: Arch. Int. Med. **31:** 164, 1923.
346. Lamarre: These Doctorat-Vétérinaire, Paris, 1933.
347. Risse: Fortschr. a. d. Geb. d. Röntgenstrahlen. **31:** 148, 1923.
348. See quoting article. (*Note:* Any reference with this number following it indicates that quoting author failed to include reference.)

THE EFFECT OF OPERATOR ON THE VARIABILITY OF BASAL METABOLISM DATA

DENA CEDERQUIST,* MANHATTAN, KAN.

STUDIES of basal metabolism reported in the literature suggest that all tests were made under standard conditions. Standard conditions for the determination of basal metabolism have come to mean that the person was in a postabsorptive state, comfortably warm, had a normal body temperature, was awake but at complete rest and free from the effects of physical exercise, mental and physical irritation, and fear. As it is almost always possible to meet these conditions, the error associated with technical procedure would be expected to be reduced to a minimum. Thus two successive observations made on one person under the foregoing conditions should agree closely, or in the language of the statistician, the two observations should show little intradaily variation. However, in actual practice, more or less variation tends to occur.

In this laboratory 450 tests on 225 women between 15 and 45 years of age were made under what were believed to be standard conditions. A Benedict-Roth closed circuit machine was used. In most cases the subjects were brought to the laboratory by car and were required to rest in bed one-half to one hour before the observations were made. A few of the tests were made in a women's residence hall. For these, the metabolism apparatus was moved into the subject's room, and the observations were made in the morning before she arose. Two eight-minute tests with a three- to five-minute rest period between them were made on each person. All observations were used unless some obviously disturbing factor, as an unexpected noise, affected the test. Five technicians were responsible for collecting these data.

Because the observations were made under standard conditions using the same equipment, it would seem logical to expect that the variation observed in individual records made on the same day would be of the same magnitude for the entire group. To determine if this were true, an analysis of variance was made on the 450 tests with the records divided on the basis of the technician who made the test. That portion of the analysis relative to the variation observed in records made on the same day is shown in Table I.

In the interpretation of this portion of the analysis of variance, the size of the mean square is used as a measure of intradaily variation. The larger the value of the mean square, the greater is the variation in the observations made on one person on any given day. It can be seen from Table I that this variation,

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as indicated by the value of the mean square, is not the same for all technicians. It will be noted that for Technician O the mean square of the records on the same day is about two and one-half times that of Technician B. This difference is great enough to be of statistical importance. It indicates that successive tests made by Technician O show significantly more variability than those made by Technician B. This observation has suggested that in this study, where the same piece of apparatus was used each time under what is believed to be standard conditions, some of the differences in the amount of intradaily variation may be attributed to the technician.

TABLE I

ANALYSIS OF INTRADAILY VARIATION

TECHNICIAN	DEGREES OF FREEDOM*	MEAN SQUARES†
B	62	1.42
C	232	1.61
R	42	2.17
K and C	124	2.18
O	48	3.48

*Degrees of freedom = (n-1) where n refers to the number of observations.

†Mean square or variance = $\frac{\text{sum of the squares of deviation from mean}}{\text{degrees of freedom}}$

No definite explanation for the differences noted between technicians is offered, but a plausible suggestion is that they may be due to differences in sensitivity of the observer. For example, one technician might note every unexpected noise, draft, or bright light which could disturb her subject and would discard the test because it was not done under standard conditions. Another operator might not recognize such disturbing factors and would believe that standard conditions had been established when in reality they had not. Also, not all persons approach a given situation with the same degree of relaxation. If the operator is tense, it is possible to induce a tension in the subject without the latter realizing she has been disturbed. In either case a source of error has been introduced which will increase intradaily variation.

Part of the intradaily variability in basal metabolism tests on the same person may often be attributed, therefore, to some characteristic of the technician. When all technicians are carefully trained, the differences in variability cannot be attributed to careless workmanship, but may be accounted for by differences in sensitivity to the interpretation of standard conditions. This study suggests that, even though standard methods have been outlined for the determination of basal metabolism tests, it is possible for the technician to introduce variability and thus decrease the reliability of the tests.

LABORATORY SHAKING MACHINE*

E. E. Myers, M.D., Philippi, W. Va.

A STURDY, silent shaking machine, which can be easily built in a small machine shop, is shown in the photograph. The diagram gives detail of construction. The length and period of excursion are those specified for use in the Kahn test. With used connecting rods and a used motor the machine can be built for twenty-five to thirty dollars.

Fig. 1 in the diagram is a side view of the shaking machine. The rocking arms are model A Ford connecting rods with the crankshaft-bearing portions cut off. They are welded together to make an overall length of $10\frac{7}{16}$ inches. The wrist pins must be parallel; this can be accomplished by a wooden jig that has parallel blocks on which the pins are held by clamps during the welding.

The wrist pins are clamped to the baseboard and top board by $\frac{3}{4}$ inch pipe clamps, the boards being hollowed slightly to allow the connecting rod parts which extend beyond the wrist pins to have freedom of movement. Side motion is prevented by the lock rings in the connecting rods. The oil holes on the bottom pieces are plugged with wooden plugs. Celluloid cement can be coated over the ends of the plugs to prevent seepage of oil through the paint. The oil hole at the top is left open to receive oil (about number 10 motor oil is used for lubrication). All wrist pins should be a medium tight fit only.

The throw arm is made from two V-8 Ford connecting rods with a short piece of model A rod welded between them to give a total length of $12\frac{1}{4}$ inches from center to center. The wrist pins of the throw arm are cut off just the width of the throw arm plus the thickness of two fiber washers which go one on either end of the pin. Fig. 7 shows another view of the connection of the throw arm to the rocking arm. The $\frac{3}{8}$ inch bolt is drawn up tightly, holding the wrist pin securely against the surface of the rocking arm, which, where the pin strikes it, is filed to a flat surface. The fiber washers shown as *F* in Fig. 7 then act as spacers for the throw arm, eliminating noise. The connection of the throw arm to the eccentric is made in a similar manner. The plate for the eccentric is shown in Fig. 6. The holes in it must be parallel, and after it is welded to the shaft of the bearing shown in Fig. 5, the surface to which the throw arm is connected should be machined in a lathe to give a flat surface parallel to the throw arm. This is essential to prevent binding of the throw arm during rotation of the eccentric. The $\frac{3}{8}$ inch machine bolts used to fasten the throw arm bearings are somewhat smaller than the holes in the wrist pins through which they pass, and this permits adjustment of the arm on the eccentric to produce the exact excursion of the top board of $1\frac{1}{2}$ inches.

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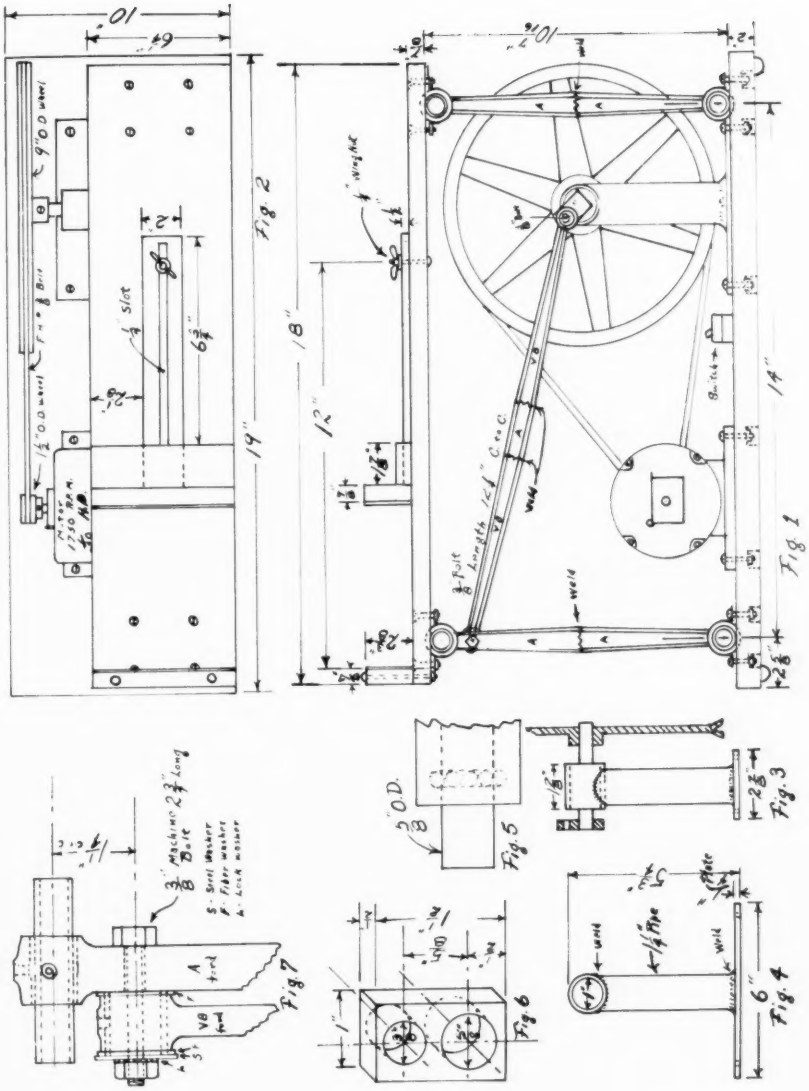

Figs. 1-7.

The bearing illustrated in Figs. 3 and 5 is an automobile packless type water pump ball bearing and shaft, New Departure, No. 885156S, 108556. While welding the plate to the shaft of this bearing, care must be taken to prevent the bearing from becoming too hot, as by wrapping wet asbestos paper around it. Electric welding is necessary here. The bearing case is pressed into a piece of 1 inch pipe coupling which can be used without alteration, i.e., the threads are left intact. Before the bearing is fitted, the coupling is welded to a piece of 1¼ inch pipe, as shown in Figs. 3 and 4. This pipe is welded to a plate which forms the base to be attached to the bottom board by four bolts.

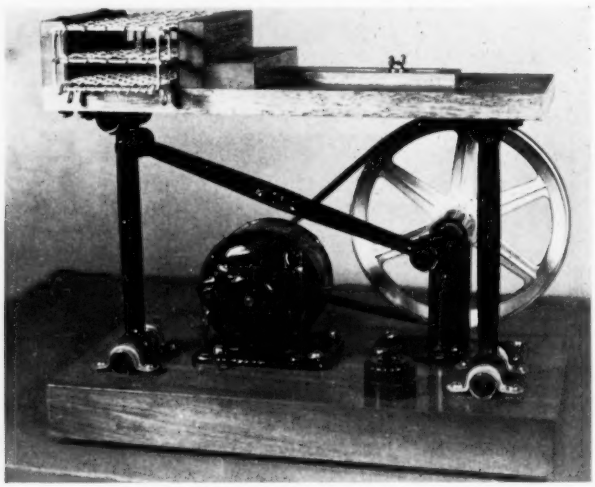

Fig. 8.

Fig. 8 is a top view of the completed machine. The boards forming the clamp for the Kahn racks are covered with rubber cut from stair tread. These rubber pieces can be cemented or tacked with small brads. Carriage bolts ¼ by 3½ inches are used to attach the end board. A block with a V in it can be made for holding a bottle, permitting it to be clamped in place of a rack.

The motor is a 1,750 r.p.m. and 1/30 h.p. (or larger). Using a 1½ inch pulley on the motor, the speed will be slightly slower than that required. The speed can be increased by wrapping tire tape around the pulley in a direction opposite that of its rotation. The speed is checked after each addition of one or two layers of tape until the revolutions per minute of the large wheel, as determined by a speed indicator (or by oscillations of the top board), are between 275 and 285.

Diameters of pulleys are 1½ inches and 9 inches. The pulleys may be obtained from Central Die Casting and Manufacturing Co., Chicago, as 150A and 900A, respectively. Length of belt is 33½ inches. It is a V belt, Goodrich, FHP No. 1340.

A METHOD FOR MEASURING THE TENSILE STRENGTH AND STRETCH OF PLASMA CLOTS*

I. M. Tarlov, M.D., Alvin I. Goldfarb, M.D.,† and Bernard Benjamin, M.D.
Brooklyn, N. Y.

IN THE course of experiments on autologous plasma clot suture of nerves,[1] a somewhat crude but simple method of testing the tensile strength and stretch of clots formed in vitro was devised. This technique has since been improved and in its present form has given fairly consistent measurements which have aided in the determination of the most suitable types of plasma clot for use as nerve suture material. Since the method may be of use in other fields of investigation, particularly the hematologic, a description of it is considered advisable. Careful search of the literature has disclosed previous methods for measuring the tensile strength and stretch of plasma clots.[2, 3] However, the present method, which was independently evolved, appears simpler, more readily constructed, and seems to us more suitable for accurate determination of these values.

Blood is drawn in a chilled syringe coated with mineral oil and is immediately transferred to paraffin-lined test tubes packed in ice. The tubes are placed in 250 c.c. metal cups filled with ice and are centrifuged for from five to seven minutes at 2,750 r.p.m. Centrifugation for longer periods often results in clotting of the plasma. On removal from the centrifuge the tubes are at once repacked in ice, and the supernatant unmodified plasma is ready for use. If blood to which an anticoagulant has been added is used, it is chilled and centrifuged in an identical fashion.

One cubic centimeter of the plasma is pipetted into a test tube of 8 mm. inside diameter in a water bath at 37° C. and allowed to clot. When a clotting agent is used, it is placed in the test tube in the water bath for a brief interval before addition of the plasma so that the temperatures may be as constant as possible throughout. After coagulation the clot is withdrawn from the tube by touching the tip of a heated platinum wire to the upper surface. The clot adheres to the wire and can easily be lifted out. When clot retraction has not occurred and the clot adheres to the test tube, a fine cool platinum wire is run carefully around the clot to loosen it.

The upper end of the clot is grasped in the rubber-sleeved jaws of a small hemostatic clamp held horizontally by means of a support, clamp holder and clamp. A common pin is pushed through one of the rubber sleeves and points to the zero mark of a millimeter rule held vertically by another clamp affixed

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†Fellow in Surgical Research.

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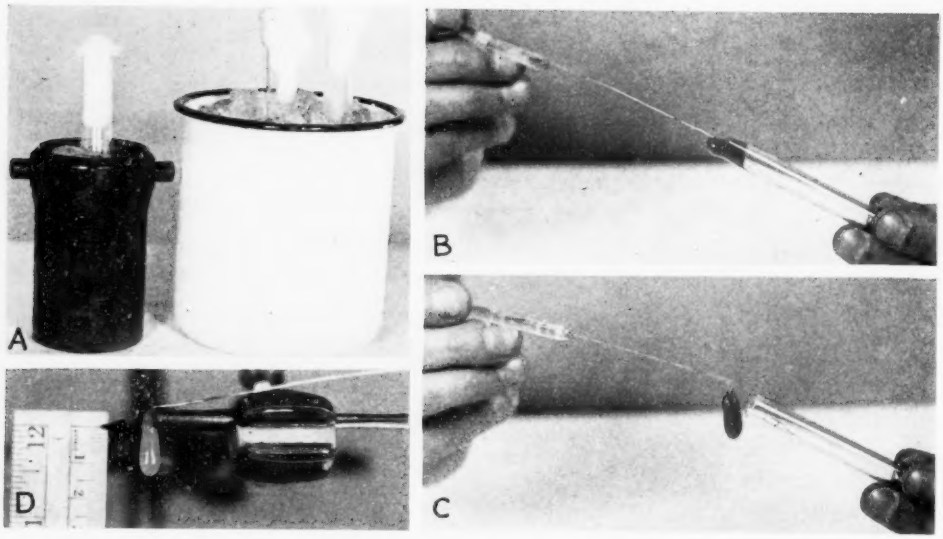

Fig. 1.—*A*, Metal centrifuge cup and enamelware pot containing test tubes packed in ice; *B* and *C*, removal of plasma clots from test tubes by heated platinum wire; *D*, grasping clot in clamp.

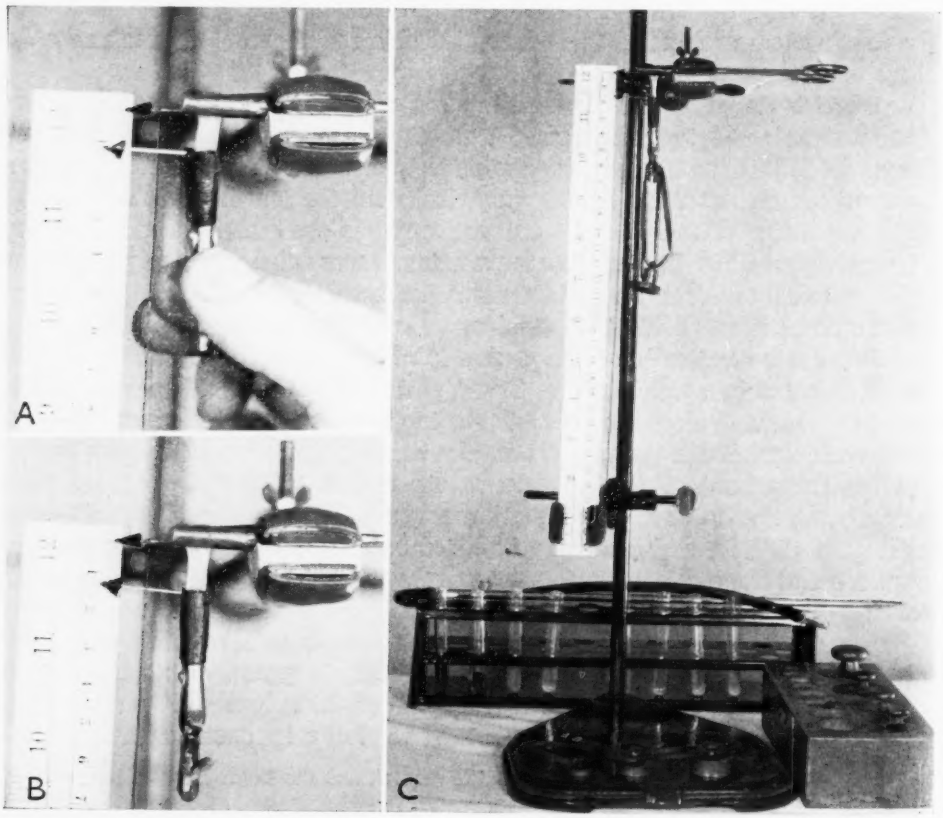

Fig. 2.—*A*, Application of clip to clot, leaving 1 cm. free for testing; *B*, stretch of 1.5 mm. following application of clip; *C*, use of apparatus for measuring tensile strength and stretch of clots. Clot shown supports clip and weights totaling 15 Gm. with stretch of 4.5 mm.

to the same support. The rule is just behind and to the side of the clot. The end of the pin pointer is fixed to the zero mark of the rule with a drop of collodion. This zero mark is permanently adjusted to correspond to the junction of the clot and clamp (Fig. 1). A light "radio clip" with the cylindrical wire guide cut open and bent to form a hook and with rubber-sleeved flattened jaws is clipped to the lower end of the clot. One centimeter of free clot is left for testing. Through one of the rubber sleeves of the clip is another common pin which indicates a mark on the rule level with the junction of clot and clip. A heavy silk thread is knotted about the upper pointer, strung parallel to the rule, and fixed to the clamp below. This serves as a guide for the lower pointer which runs between the rule and the thread and also serves to damp the swing of the clip which results from the addition of weights. The stretch resulting from the application of the clip (weight 4.0 Gm.) and each additional weight load is measured as the increase in distance between the two pointers. Rubber bands (weight 0.5 Gm.) are slit so as to slip over the heads of weights, and the weights are thus gently added to the hook of the clip at intervals of fifteen to forty-five seconds until the clot breaks (Fig. 2). It was found convenient to remove all the weights at 40 or 45 Gm. and to substitute for them, adding the next increment, a single 50 Gm. piece. At this point the tendency of the clot to approximate its original length is recorded. This may be repeated at 90 to 95 Gm. by adding a single 100 Gm. piece after removal of the weights. A cotton padded drinking cup may be placed beneath the weights to catch them and the clip when the clot breaks. The tensile strength of plasma clots from 8 healthy adult human males was found to average 44 Gm. The values obtained from ill persons were frequently considerably higher. The stretch seems to be directly proportional to the weight load; each 10 Gm. increment causes an average stretch of 2 to 3 mm. The final increment causes a greater rapid stretching which results in the break of the clot. Further detailed studies of tensile strength and stretch are being carried out.

REFERENCES

1. Tarlov, I. M., and Benjamin, B.: Autologous Plasma Clot Suture of Nerves, Science **95**: 258, 1942.
2. Fonio, A.: Weiterer Beitrag zur Methodik der Untersuchung der Blutgerinnung, Schweiz. med. Wchnschr. **51**: 146, 1921.
3. Kristenson, A.: Untersuchungen über die Elastizität des Fibrinkoagulum, Acta med. Scandinav. **77**: 351, 1932.

METHOD OF SUSPENDING BOTTLE*

E. E. Myers, M.D., Philippi, W. Va.

AN EASY method for suspending an inverted bottle as for use in a Wangensteen suction apparatus,[1] etc., is described.

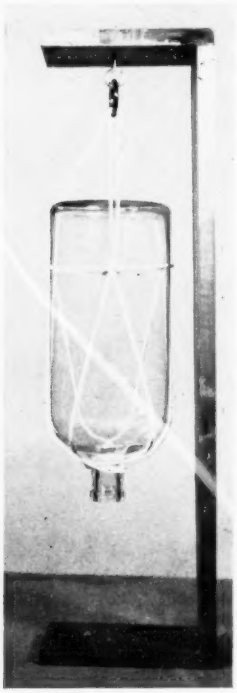

Fig. 1.

Either Venetian blind cord or suspender cord is satisfactory. A piece about 3 feet in length (for a gallon bottle) is used to make a ring around the bottle with three loosely tied knots evenly spaced. A second piece of cord, about 10 feet long, is started down through one of these loops, brought around the neck of the bottle, crossed over and brought back up through the next loop. It goes through the iron ring (harness ring 1¼ inches in diameter) which is to suspend the bottle, passes down through the third loop and continues as above until there are two ropes through each loop. The ends of the rope are tied together and the knots in the rope around the bottle are drawn tightly.

Fig. 1 shows the appearance of the completed suspension.

REFERENCE

1. Myers, E. E.: An Easily Constructed Wangensteen Suction Apparatus, J. A. M. A. 116: 591, 1941.

*From the Myers Clinic, Philippi.
Received for publication, March 30, 1942.

PROTECTIVE CAPS FOR GLASS TISSUE GRINDERS*

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TISSUE grinders consisting of paired tubes one within the other in which tissue fragments or bacterial clumps are emulsified are widely used in virus and bacteriologic work. The tubes are roughened and are made to fit snugly by grinding with abrasive powder.

Two types are principally used: (1) The TenBroeck grinder† made of heavy pyrex glass, and which is designed mainly for manual manipulation, and (2) those more or less similar in design to that described by Hanks, the plungers of which are activated by a mechanical rotary device.[2-4]

The use of grinders is accompanied by the risk of contamination of the material being ground by air-borne bacteria and of contamination of the operator with the material through splashing, etc.

The apparatus of Corper and Cohen[3] and that of Hanks[4] have devices to reduce the chances of contamination. The present report is concerned with a simple means of preventing contamination of the material and of protecting the operator when either the TenBroeck or Hanks type of grinder is used.

Devol gum rubber caps, such as are used for nursing bottles or centrifuge tubes, are selected which will fit over the mouth of the receptacle tube. A hole is punched in the center of the cap with a cork borer and the shank of the plunger is pushed through it. The hole should be of such size that the shank of the plunger fits snugly but without binding.

The grinder parts are wrapped separately for sterilization. The receptacle portion is plugged with cotton and may be dry sterilized. The plunger part, because of the rubber cap attached, must be autoclaved. After inserting the material to be emulsified, the lip of the receptacle tube is flamed, the plunger is inserted, and the cap is fitted over the outer tube with aseptic precautions.

The use of the protective cap is advantageous in grinding tissues by hand, since considerable manipulation for a time may be necessary and abundant opportunity for contamination is present.

Likewise, a protective device is desirable when cultures or tissues are emulsified using a mechanical rotor. The gum rubber caps described are more convenient than the holed cork stoppers used by Hanks, since diluting fluids can be aseptically introduced through the cap without stopping the motor by means of a syringe and hypodermic needle after rubbing the surface with alcohol.

REFERENCES

1. TenBroeck, C.: A Simple Grinder for Soft Tissues, Science **74**: 98, 1931.
2. Coria, N. A.: Personal communication, 1935.
3. Corper, H. J., and Cohen, M. L.: A Mechanical Device for Preparing Fine Suspensions of Tubercle Bacilli and Other Microorganisms, J. LAB. & CLIN. MED. **21**: 428, 1936.
4. Hanks, J. H.: A Grinder for Homogenizing Bacterial Clumps or Infected Tissues, Science **94**: 615, 1941.

*From the Laboratories of Bacteriology, University of Notre Dame, Notre Dame.
Received for publication, April 7, 1942.
†Obtained from Scientific Glass Apparatus Co., Bloomfield, N. J.

A SIMPLE TEST TUBE RACK AND COMPARATOR*

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THE rack for test tubes is very simple and space saving, as can be seen from Fig. 1. If a test tube without a lip is desired, the lip can be easily substituted by a rubber band placed around the top of the tube. Should it be necessary to observe the lower part of the test tubes for extended periods of time, a detachable block may be placed to support each end of the rack (Fig. 2).

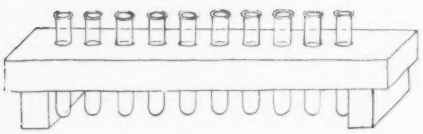

Fig. 1.

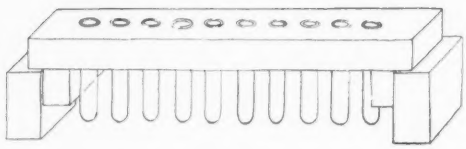

Fig. 2.

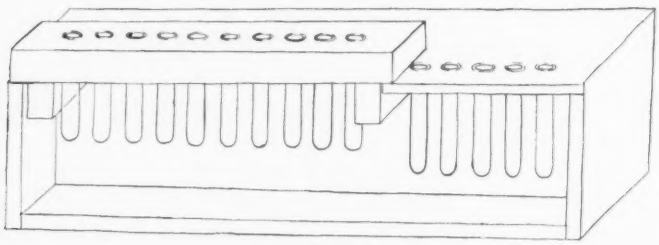

Fig. 3.

Further, the rack can be used to complement a light box for comparative readings and thus is a part of a simple comparator for color intensity or for measuring the brightness of light reflected by the particles in suspension in a tube. It was used in our studies with the toluidine blue reaction for heparin.[1, 2] The interior of the light box is painted white and contains two fluorescent bulbs of sufficient length to fill the longitudinal axis of the box. Their light is reflected through a plate of milk glass and thus illuminates the samples under test. The box can be used also as a titration light.

Fig. 3 illustrates the comparator with a permanent rack for tubes containing standard solutions and a support for the sample rack described above. Both rack and comparator are constructed from wood and are, therefore, inexpensive to manufacture.

REFERENCES

1. Copley, A. L.: Science **93**: 478, 1941.
2. Copley, A. L., and Schnedorf, J. G.: Am. J. Physiol. **133**: 562, 1941.

*From the Hixon Laboratory for Medical Research, University of Kansas.
Received for publication, April 3, 1942.

AN APPARATUS FOR THE SIMULTANEOUS MEASUREMENT OF INTRAMUSCULAR AND VENOUS PRESSURE*

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LOS ANGELES, CALIF.

THE use of Henderson's modification of the instrument devised by Kerr and Scott[1] for the measurement of intramuscular pressure has certain drawbacks. (a) It requires the use of two men, both of whom must be trained in its use. (b) Especially difficult and a source of most errors is the reading of the end point when the meniscus begins to move in the capillary tube. (c) The need to refill constantly the capillary tube with sterile saline when frequent readings are desired.

Principally, the shortcoming of this method is the need for manpower greater than our team could furnish when simultaneous venous pressures were desired.

Tests conducted with the Henderson apparatus and our modification of the instrument by Wells, Youmans, and Miller[2] convinced us that the macroscopic gravity method for the measurement of the intramuscular pressure gave comparable results.

For convenience, the venous manometer and the intramuscular pressure manometer are mounted together. The sections can easily be disengaged for sterilizing (Fig. 1). The reservoir (R_{IP}), which holds 50 c.c. saline, is connected through a glass stopcock (S) and a T tube to a 5 mm. glass tube with a 3 mm. bore (M). The outlet is controlled by a screw clamp (Sc) over a rubber tube which holds the female portion of a metal-connecting link (C). The male portion is inserted into rubber tubing, which in turn is connected to a needle adapter, and the 26 gauge needle which is inserted into the muscle.

TABLE I

INTRAMUSCULAR AND VENOUS PRESSURE READINGS OVER A PERIOD OF 60 MINUTES

PATIENT	TIME (MIN.)	INTRAMUSCULAR PRESSURE (MM. H_2O)	VENOUS PRESSURE (CM. H_2O)
D	0	82	6.9
	7	84	6.6
	12	80	6.7
	17	80	
	20	74	
	30	77	7.9
	35	73	8.1
	45	73	7.9
	60	73	8.2

*From the Cedars of Lebanon Hospital, Los Angeles.

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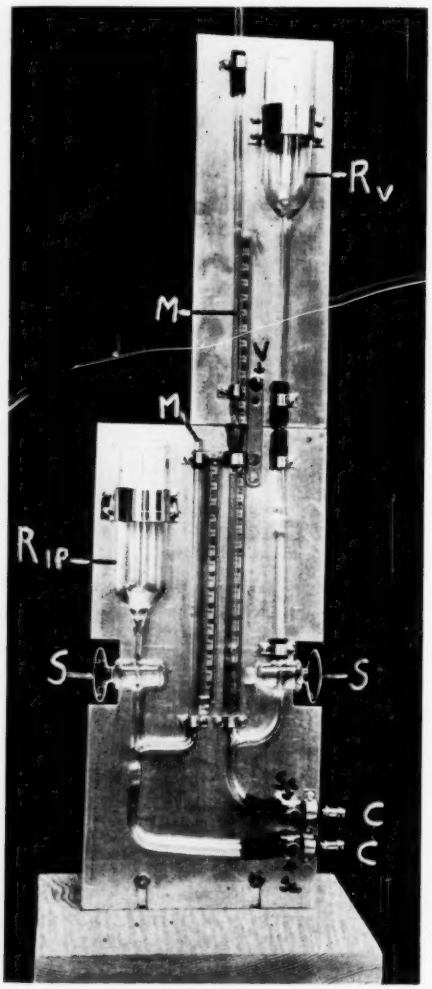
The venous manometer is similarly constructed, but the tubing of the funnel and the manometer is split to facilitate sterilizing. The manometer and the reservoir tubes are connected by standard glass or metal-connecting links (Fig. 2).



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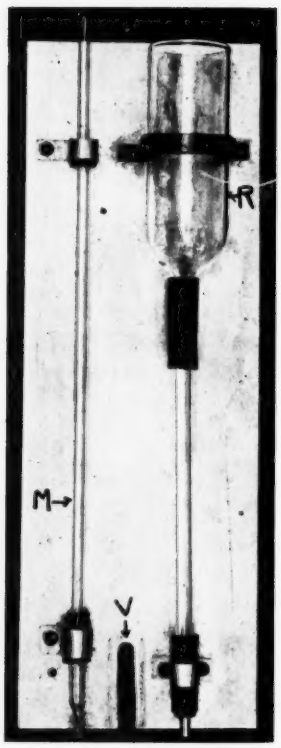

Fig. 2.

Fig. 1.—Apparatus for the simultaneous measurement of intramuscular and venous pressure, assembled after sterilizing.

Fig. 2.—Upper part of apparatus removed to show glass and metal connections and slot "V."

A vertical piece of metal (*V*) is mounted front and back to reinforce and give rigidity to the extension piece which is slotted (Fig. 2) to slip over the screw that is tightened lightly when the apparatus is assembled.

The venous reservoir (*Rv*) is filled with 3 per cent sodium citrate and the venous pressure needle is gauge 18.

The intramuscular reservoir (R_{IP}) is filled with saline. The special slotted needle described by Henderson[1] is inserted into the muscle. The zero level of

the apparatus above the floor is determined by means of a water air bubble level, attached to a stiff steel tape (Fig. 3).

The level of the needle in the vein and in the muscle and the level of the auricle are obtained by the same means. The manometer tubes are filled from the reservoir, and are permitted to attain their level by gravity. It takes about five minutes for the fluid to equilibrate with the muscle and two to three minutes in the vein. Thus continuous readings of the intramuscular and venous pressures can be made at five-minute intervals.

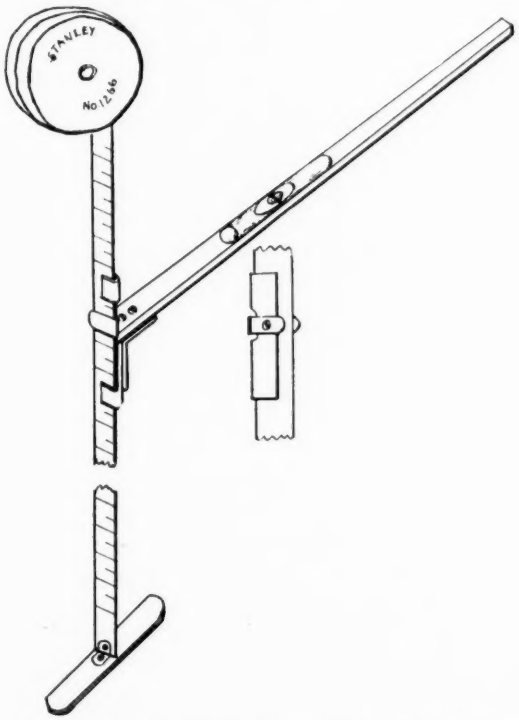

Fig. 3.—Steel tape and water level arrangement for obtaining zero point of apparatus, auricular level, and levels of the intramuscular and intravenous needles.

Difficulty with the reading may be had with the first intramuscular pressure reading. It may be necessary to pull the needle back or to insert it deeper to obtain a free fall of the fluid level in the manometer. A second and a third check reading will ascertain the workability of the apparatus. Thereafter, readings may be obtained over a long period of time merely by refilling the manometer tube from the reservoir to a level above the value of the intramuscular pressure and allowing it to fall by gravity. Likewise, the vein is flushed with 3 per cent sodium citrate from time to time to keep the needle clear, and continuous readings may be taken at will.

It will be found that intramuscular pressure readings will vary over a range of 10 mm., and venous pressure readings over a range of 2 cm. in any series of readings (Table I). The latter will vary only 1 cm. if the patient is quiet, not straining or coughing, but will vary over 2 cm. range if the patient is apprehensive.

REFERENCES

1. Henderson, Y.: Adventures in Respiration, Baltimore, 1938, Williams & Wilkins Co., page 243.
2. Wells, H. S., Youmans, J. B., and Miller, D. G.: Tissue Pressure (Intracutaneous, Subcutaneous and Intramuscular) as Related to Venous Pressure, Capillary Filtration and Other Factors, J. Clin. Investigation **17**: 489, 1938.

AN IMPROVED ROUTINE TISSUE STAINING TECHNIQUE FOR FORMALIN-FIXED TISSUES*

HENRY S. BLANK, A.B., M.D., COLUMBIA, S. C.

AS A MATTER of convenience, time, and economy the time-honored routine tissue stain has been that of hematoxylin and eosin, almost to the exclusion of any other. The technique is simple, even for inexperienced hands; the results are adequate for routine diagnosis of surgical and necropsy pathology. The stain has its limitations, however, particularly in the presentation of certain histologic and pathologic structures. The chromatic expression of these structures in stained sections has given rise to a host of special stains which are individually applicable. Lack of time, material, and technical assistance has limited the use of these techniques. This is especially true of smaller pathologic laboratories. It follows that any technique that will give better and more extensive detail with a minimum of complexity will be welcomed.

Of the several other generally advocated routine tissue staining techniques, the Mallory eosin or phloxine methylene blue procedure has few peers. However, the effective presentation of histologic detail by this method is beset by several difficulties. Among these are the more or less axiomatic requirement of Zenker fixation, length of staining time, individual differentiation of slides under the microscope, and finally, lack of uniformity of results except in expert hands.

It was the aim of this laboratory to effect a staining technique that had the advantages of the methylene blue eosin system with none of its disadvantages.

GENERAL PRINCIPLES

Ohlmacher's discovery of the mordanting action of formaldehyde on coal tar dyes was utilized in the preparation of a methylene-blue-azure mixture.[1] Giemsa stain was added to the methylene blue as a source of the azures. It appears to contribute a generally wider chromatic scale by giving additional variation in the shades of blue.

Mercurochrome 220, commonly used as a disinfectant, replaced eosin. This dye is dibrom-oxymercury-fluorescein and is closely related to eosin which is tetra-bromfluorescein. Conn[2] quotes Baldwin as stating that it may be used in

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Published with permission of the Medical Director, Veterans' Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

2 per cent aqueous solution in place of eosin for tissue work after Zenker fixation. Conn states that it has a stronger affinity for cytoplasmic structures than eosin and staining properties not unlike erythrosin or phloxine.

It was determined that the 2 per cent aqueous solution gave far too brilliant results, and that a dilution of 0.1 per cent was more than adequate. The stain has an apparent specific affinity for cytoplasmic structures and is not easily washed out. The diffuseness of staining, so characteristic of eosin, is quite absent.

REQUIRED STAINS

1. Hematoxylin, Delafield.
2. Methylene blue—0.5 Gm. dissolved in 10 c.c. of absolute alcohol diluted to 100 c.c. with 4 per cent neutral formalin.
   Giemsa stain—stock solution—4.5 c.c. diluted to 100 c.c. with 4 per cent neutral formalin.
   Mix both stains. This mixture forms a stock solution to which is added 1 Gm. of borax. Dilute 1:20 for use.
3. Mercurochrome 220—2 per cent stock solution 5 c.c.
   Alcohol 95 per cent 25 c.c.
   Water To make 100 c.c.

STAINING PROCEDURE

1. Deparaffinize formalin-fixed tissue sections in two changes of xylol.
2. Absolute alcohol —one minute.
3. Alcohol, 95 per cent—one minute.
4. Alcohol, 70 per cent—one minute.
5. Water —one minute.
6. Hematoxylin, Delafield—five minutes.
7. Wash in two changes of water to remove excess stain.
8. Decolorize until rose colored in water to which several drops of concentrated hydrochloric acid have been added.
9. Wash in water.
10. Wash until blue in water to which several drops of strong ammonium hydroxide have been added.
11. Wash in water.
12. Mercurochrome solution—one minute.
13. Wash in water to remove excess stain.
14. Methylene blue—azure stain—two minutes.
15. Wash in water to remove excess stain.
16. Decolorize in 95 per cent alcohol until no more color is seen to come off in drippings, about fifteen to thirty seconds.
17. Wash briefly in 95 per cent alcohol and complete dehydration in two changes of absolute alcohol.
18. Clear in two changes of xylol.
19. Mount in balsam.

It is desirable to filter the mercurochrome before using, to remove the bright golden scum which forms on standing for any length of time. A 0.1 per cent aqueous solution has been found satisfactory, without the addition of

alcohol, but the latter appears to give slightly better results. No particular improvement was noted by the addition of colophony to the differentiating alcohol.

Staining by this technique appears to give greater nuclear detail than is observed by hematoxylin and eosin. Fibrous tissue is sharply differentiated from muscle tissue. Inflammatory and blood elements are exceptionally prominent and present detail in both nucleus and cytoplasm. Other structures stain as in the Mallory technique.

SUMMARY

A new staining technique is presented which is suitable to routine tissue sections fixed in formalin. It is simple, does not require individual microscopic differentiation of sections, and produces excellent and uniform results even in inexpert hands.

REFERENCES

1. Lee, B.: Microtomist's Vade-Mecum, ed. 9, Philadelphia, 1928, P. Blakiston & Sons, page 170.
2. Conn, N. J.: Biological Stains, ed. 4, Geneva, N. Y., 1940, Biotech Publications, p. 151.

A NEW DEVICE FOR THE APPLICATION OF SCALP ELECTRODES IN ELECTROENCEPHALOGRAPHY

ROBERT COHN, M.D., WASHINGTON, D. C.

THE device consists of a spring air valve seated in a brass or plastic casing. Fig. 1 shows in longitudinal section the essential design characteristics and mechanism of the device. The total weight of the electrode applier, with brass casing, is less than 15 Gm.

METHOD OF USE

The scalp surface is prepared according to standard procedures. The solder pellet electrode is then held in position by placing the valve pin on the electrode and making gentle pressure on the thumb rest of the electrode applier. A drop or two of collodion is then allowed to flow over the electrode. To hasten the setting of the collodion, it is necessary to disperse its volatile solvents. This is readily accomplished by a blast of air from the device. A moderate increase in the pressure exerted on the thumb rest of the electrode applier opens the valve and allows the compressed air to escape through the applier outlet. Three to five pounds per square inch of pressure is quite adequate for efficient drying. Since only low pressures of air are required, the electrode applier can be con-

*From the Laboratories of St. Elizabeths Hospital, Washington, D. C.
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nected to the compressed air-reducing valve (from tank or line) by means of thin-walled rubber tubing. This adds much to the general ease of manipulation of the device.

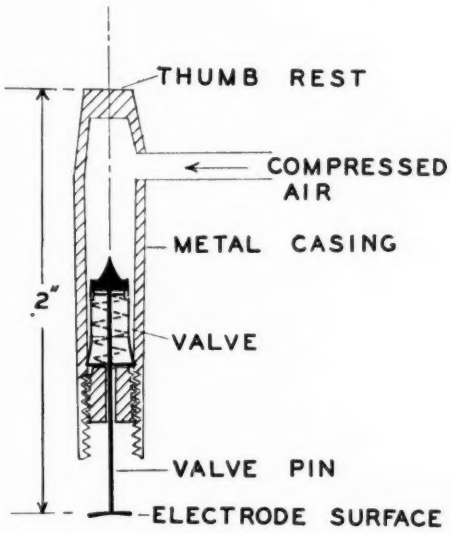

Fig. 1.

CONCLUSIONS

This electrode applier simplifies the technique of electrode application for the following reasons:

1. Ease of manipulation resulting from its compactness and lightness.
2. Efficiency of operation.
3. More firm and more uniform application of electrodes result from the minimum pressure required to open the air valve.
4. Rapidity of electrode application because of the proximity of the air blast to the applied electrode.

CHEMICAL

SIMPLIFIED OXYGEN ANALYZER FOR OXYGEN TENTS*

A. H. Andrews, Jr., M.S., M.D., and L. W. Roth, Ph.D.
Chicago, Ill.

THE importance of oxygen concentrations in the therapeutic uses of oxygen has received considerable emphasis. In spite of this, many oxygen tents are not controlled by oxygen concentration determinations.

Many oxygen tent analyses were made and the majority of the determinations showed oxygen concentrations to be from 30 to 45 per cent. After the nurses were instructed to flood the tents more frequently, the concentrations were somewhat higher but were still not satisfactory. It was also apparent that regulation of the concentration by a technician to 50 per cent or over three times a day would not maintain the concentrations satisfactorily between his visits because of the nursing care which necessitated opening the tents. It was concluded, therefore, that oxygen analysis by the nurses was necessary in order to maintain the desired concentrations.

The following criteria were considered essential for an oxygen analyzer suitable for use by nurses: 1. Use of equipment familiar to nurses. 2. No adjustment of fluid level. 3. Absence of valves. 4. Reasonably foolproof. 5. Inexpensive. 6. Accuracy within plus or minus 2 per cent.

The analyzer described below was devised. The fifteen which were built and used for two years with satisfactory results fulfilled the above-mentioned criteria.

DESCRIPTION

The analyzer consists of a 200 c.c. glass jar (see Fig. 1) filled with No. 30 pure copper cloth. A No. 11½ rubber stopper is drilled to hold a glass tube 8 mm. in external diameter and 10 inches long, and to hold firmly the tip of a standard 10 c.c. syringe. The glass tube extends from the bottom of the jar high enough so the fluid does not overflow. The lower end of the tube is constricted by heating and the upper end is closed with a small toy balloon.

The scale consists of a strip of paper cemented to a strip of light brass and held on the tube with rubber bands. The scale is calibrated by injecting with the syringe the following amounts of air, and after the fluid stops dropping, the level is marked at the concentration as indicated: 10 c.c. of air, 21 per cent; 8.75 c.c., 30 per cent; 7.5 c.c., 40 per cent; 6.25 c.c., 50 per cent; 5 c.c., 60 per cent; and 3.75 c.c., 70 per cent.

The solution in the copper-ammonia-chloride reagent, recommended by Badger,[1] consists of equal amounts of water and concentrated ammonium hy-

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droxide (28 per cent) saturated with ammonium chloride. This solution does not give off enough ammonia to be objectionable. As it becomes exhausted, the solution forms a yellow precipitate which can be dissolved with a small quantity of the fresh solution.

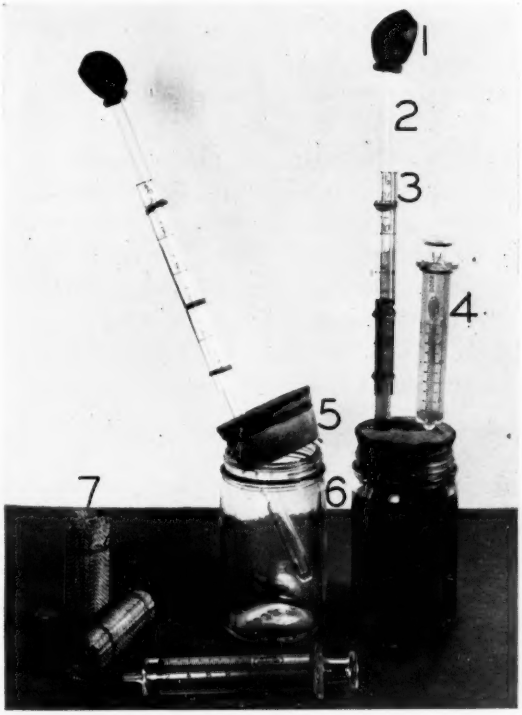

Fig. 1.—Simplified oxygen analyzer. *1*, Toy balloon; *2*, glass tube; *3*, scale; *4*, syringe; *5*, rubber stopper; *6*, glass jar; *7*, roll of copper cloth.

TECHNIQUE

A 10 c.c. syringe, moistened occasionally with water, is attached to the oxygen sampling outlet or catheter extending into the tent and flushed four to six times. The plunger is withdrawn to the 10 c.c. mark, the syringe is detached, and the tip is covered with the finger. The tip is inserted into the hole in the rubber stopper, care being taken not to let the plunger move during the procedure. The plunger then is pushed down gently and twisted until it sticks. After the fluid level in the tube is stationary (within twenty seconds to one minute), the concentration is read on the scale. The syringe is left in place until the next analysis.

Once every day or two the scale is checked by an injection of 10 c.c. of air. If the level does not stop at the 21 per cent mark, the scale is adjusted as necessary. This, like the replacing of the solution, should be done by a technician or by the supervisor of the oxygen therapy.

DISCUSSION

An oxygen tent is no better than the oxygen concentration. Oxygen administration is similar to administration of any medicine, and the significance of

the dosage should be recognized and controlled. Analysis by a technician at intervals is valuable, but inasmuch as the tent may have to be opened for nursing attention between analyses, there are periods when the concentration drops.

This analyzer is designed for the nurse to use. She is instructed to make an analysis each time the tent has been opened and closed, and to adjust immediately the concentration to 50 per cent or over. In addition, the concentration is determined every hour. This frequent testing of the concentration also makes the nurse realize that frequent unnecessary opening of the tent is detrimental to the patient.

The time required for the analysis is almost negligible. Withdrawing the sample and injecting it into the analyzer requires less than thirty seconds, and the nurse can return in a minute or two and read the concentration.

The cost of the material for making the analyzer itself is small; the syringe, furthermore, is a standard hospital item. The apparatus can be made in any laboratory equipped with the necessary hole cutters and metal shears. This inexpensiveness makes it possible to have several on hand. When the solution becomes exhausted, another can be substituted.

SUMMARY

An oxygen analyzer is described, which has been designed specifically for use by nurses. The analyzer is reasonably accurate, simple to use, inexpensive, and can be made in the ordinary laboratory.

Analysis of the oxygen concentration after each time the tent is closed and at frequent intervals increases the efficiency of the therapy.

REFERENCE

1. Badger, W. L.: The Determination of Oxygen by the Copper-ammonia-chloride Reagent, Indust. & Engin. Chem. **12**: 161, 1920.

THE SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHORUS AND CHOLESTEROL*

MILDRED KAUCHER, M.S., VIRGINIA BUTTON, A.B., AND HAROLD H. WILLIAMS, PH.D., DETROIT, MICH.

RECENT advances in the development of spectrophotometric instruments for precise color measurement make it possible to attain equal or greater accuracy in chemical analysis and to employ procedures which have been hitherto unavailable. Furthermore, they offer the advantage of increasing the number of determinations that may be performed in a given time. This paper presents the adaptation of phosphorus and cholesterol methods to the spectrophotometer.

PHOSPHORUS DETERMINATION

The boundaries of quantitative biochemistry have been greatly extended by Van Slyke and his associates through the development and application of the gasometric technique for the microdetermination of organic carbon.[1-3] The principle of their procedure has been adapted successfully to microquantities of inorganic phosphate,[4] sulfate,[5] calcium,[6] and magnesium[7] by manometric combustion of their organic precipitates. The introduction and use of special combustion centrifuge tubes[5, 6, 8] permitting, without transfer, precipitation, washing, and combustion of the organic compound has simplified the method and expedited the time needed for carrying out the determination.

From the viewpoint of microtechnique and accuracy, the gasometric determination of phosphorus is ideal. The laboratory possessing a spectrophotometer, however, may use the present adaptation to advantage, since it facilitates with a similar degree of accuracy the analysis of large numbers of samples with less highly skilled and trained personnel, and eliminates any interference from contaminants such as atmospheric dust and carbon dioxide. Applying the following procedure, it has been possible to perform a large series of phosphorus analyses in one day whereas formerly two or three days were required.

The procedure of Kirk[4] is followed up to the washing of the precipitated phosphoric acid in the form of strychnine phosphomolybdate. An aliquot (usually 6 ml.) of the phosphoric acid sample is placed in an ordinary 15 ml. conical pyrex centrifuge test tube. Two milliliters of the strychnine molybdate are added dropwise with continuous shaking and allowed to stand not less than ten minutes.† The tube is centrifuged, the supernatant liquid is decanted, and

*From the Research Laboratory of the Children's Fund of Michigan, Detroit.
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†In the original procedure of Kirk the reagent is added rapidly to the sample and the contents of the tube are thoroughly mixed. The tube is then permitted to stand for twenty to forty-five minutes to complete the precipitation. If carried out by the above modification, there is saving in time since precipitation is complete at the end of ten minutes.

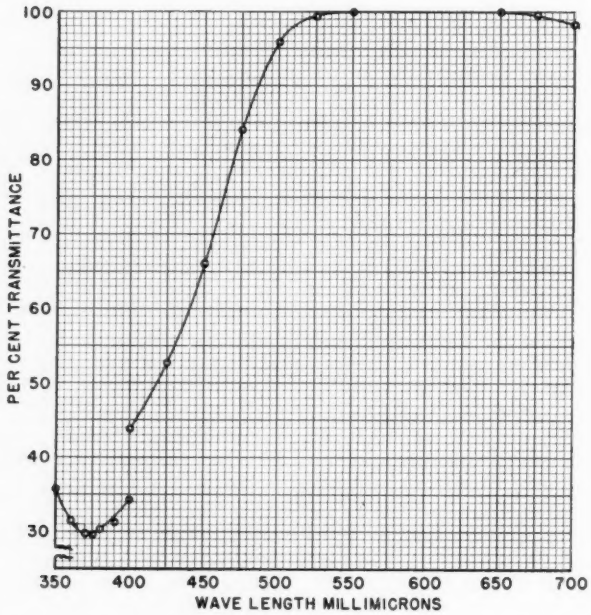

Fig. 1.—Transmittance wave length curve for color of strychnine phosphomolybdate in dilute sodium hydroxide. Filter No. PC 6 used from 350 to 400 mμ. Filter No. PC 4 used from 400 to 700 mμ. Concentration of phosphorus, 0.025 mg. in 100 ml.

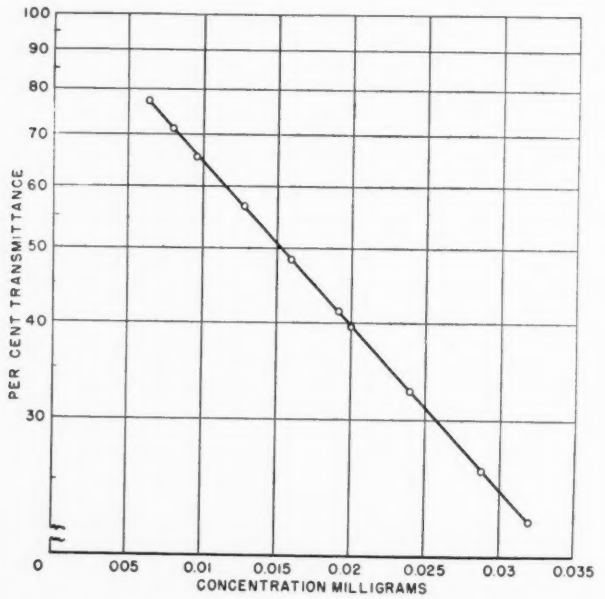

Fig. 2.—Concentration of phosphorus *vs.* transmittance.

the mouth of the tube is wiped free of any remaining liquid. Two milliliters of 1 per cent nitric acid are added, the precipitate is stirred with a glass rod, and the tube is centrifuged for five minutes. The washing with nitric acid is repeated twice (three washings in all); no stirring is necessary in the last wash. The precipitate is then dissolved in 2 ml. of 1 per cent sodium hydroxide, and

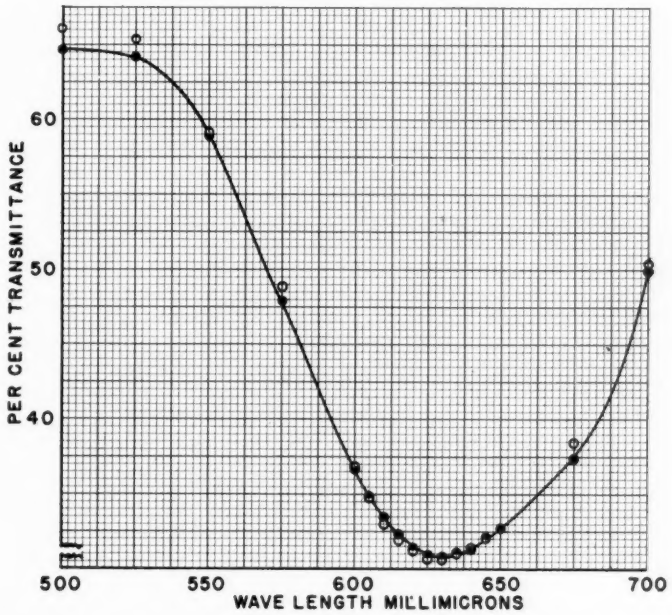

Fig. 3.—Transmittance wave length curve for color developed by cholesterol (clear circles) and cholesterol digitonide (filled in circles). Cholesterol concentration 0.6 mg. in 6 ml.

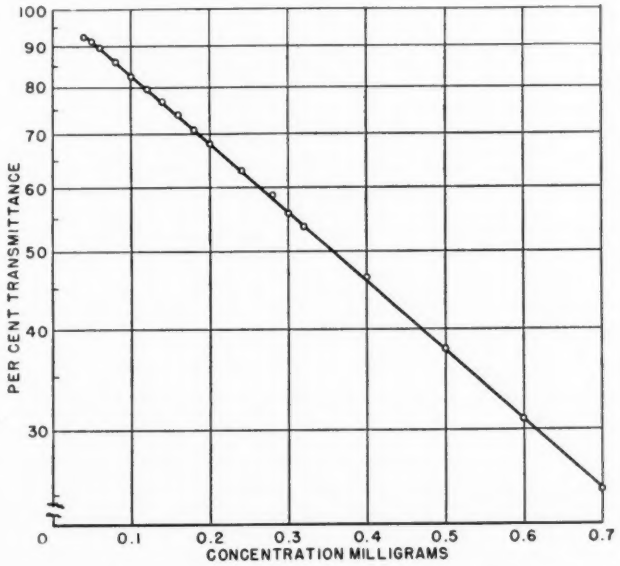

Fig. 4.—Concentration of cholesterol *vs.* transmittance. Maximum average deviation ±0.6.

the contents of the tube are completely transferred into a 100 ml. volumetric flask with distilled water. After making to volume, the sample is ready for color measurement.*

Fig. 1 shows the spectral transmittance curve of the strychnine phosphomolybdate in a dilute sodium hydroxide solution. From 400 to 550 mμ the

*In the present study the Coleman Universal Spectrophotometer was used. Any similar device that will allow for the selection of light of the proper wave length should be suitable.

absorption decreases to zero and remains there until 650 mμ is reached. Above 650 mμ there is slight absorption. Since the maximum absorption between 400 to 700 mμ was at 400, the spectral properties of the solution were examined between 350 and 400 mμ. The maximum absorption was obtained at 375 mμ.

TABLE I

COMPARISON OF SPECTROPHOTOMETRIC AND GASOMETRIC DETERMINATION OF PHOSPHORUS

PHOSPHORUS MG.	GASOMETRIC PHOSPHORUS MG.	RECOVERY GASOMETRIC PHOSPHORUS PER CENT	TRANSMITTANCE* PER CENT
0.0064	0.00648	101.3	77.0
0.0080	0.00808	101.0	71.0
0.0096	0.0098	102.2	65.3
0.0128	0.01276	99.6	56.4
0.0160	0.01588	99.3	48.3
0.0192	0.01922	100.1	41.4
0.020	0.0197	98.5	39.5
0.024	0.0244	101.6	32.6
0.0288	–	–	25.8
0.032	–	–	22.1

*Maximum average deviation ±0.9.

Table I shows the comparative values of a series of standards ranging from 0.0064 mg. to 0.032 mg. of phosphorus, as determined by both the spectrophotometric method and by the gasometric microtechnique. By plotting the transmission values against the quantities of phosphorus on semilog paper, a straight line results, as illustrated in Fig. 2, thus demonstrating that Beer's law is obeyed over the range of concentrations studied.

CHOLESTEROL DETERMINATION

There have been many procedures proposed for the determination of cholesterol. The microprocedure for the determination of free and combined cholesterol of Schoenheimer and Sperry[9] achieves a degree of accuracy which has previously been obtainable only with the classical macrogravimetric technique of Windaus. Because of the weak color intensity in the small volume required by the method, the use of this excellent and precise procedure, unfortunately, has been limited to only those investigators possessing a photometer. Most of the available photoelectric measuring devices are not equipped with cells of the proper depth to volume ratio to make accurate measurement of the limited amount of faintly colored solution.[10] The colorimeter has been employed for measuring the color, but it has not been altogether practical.[9, 10] The Coleman Universal Spectrophotometer, however, which employs a square cuvette giving a depth of approximately 1.3 cm. for a volume of 6 ml., has proved satisfactory, provided the cholesterol digitonide is dissolved in double the quantities of reagents, as recommended[9] for large precipitates.

The method up to the dissolving of the cholesterol digitonide for color measurement is exactly as given by Schoenheimer and Sperry,[9] including the more recent improvements recommended by Sperry[8, 10] The cholesterol digitonide is dissolved in 2 ml. of glacial acetic acid, 4 ml. of acetic anhydride, and 0.2 ml. of concentrated sulfuric acid. After developing the color in accordance with the original method,[9] it is measured in the spectrophotometer employing the

630 mμ wave band. From the spectral transmittance curves of cholesterol and cholesterol digitonide, as shown in Fig. 3, it may be noted that the maximum absorption occurs between 620 and 635 mμ for both compounds. Noninterference of the digitonide with the light absorption of cholesterol is a decided advantage, as was pointed out originally.[9]

Transmittance values were determined for 0.04 mg. to 0.7 mg. of cholesterol, encompassing a range higher than that given in the original method (0.02 to 0.015 mg.). This wider range has been made possible by doubling the amounts of digitonin used for precipitation of the cholesterol. The 1 ml. of digitonin solution recommended[9] will precipitate the cholesterol completely up to and including 0.3 mg. Above 0.3 to 0.7 mg., however, 2 ml. of the digitonin solution are required to precipitate the cholesterol completely. If there is doubt as to the quantity of cholesterol in an unknown, 2 ml. of the digitonin solution may be used with as low as 0.1 mg. of cholesterol. A series of samples containing from 0.1 mg. to 0.3 mg. gave the same per cent transmittance values when precipitated with 1 ml. of digitonin solution as with 2 ml. Plotting the transmittance values against the concentration of cholesterol (Fig. 4), it may be noted that Beer's law operates over the range of concentrations studied.

SUMMARY

The gasometric phosphorus and the colorimetric cholesterol digitonide methods have been adapted successfully to use with the spectrophotometer without sacrificing original features of microtechnique, specificity, and accuracy. Phosphorus may be determined within the range of 0.006 to 0.3 mg., quantities similar to the gasometric method. For cholesterol it has been possible to enlarge the quantities (0.04 to 0.7 mg.) determined without loss of accuracy. As a result of these adaptations the above micromethods should find a wider application.

REFERENCES

1. Van Slyke, Donald D., Page, Irvine H., and Kirk, Esben: A Manometric Micromethod for Determination of Carbon in Organic Compounds, J. Biol. Chem. **102**: 635, 1933.
2. Kirk, Esben, Page, Irvine H., and Van Slyke, Donald D.: Gasometric Microdetermination of Lipids in Plasma, Blood Cells, and Tissues, J. Biol. Chem. **106**: 203, 1934.
3. Van Slyke, Donald D., and Folch, Jordi: Manometric Carbon Determination, J. Biol. Chem. **136**: 509, 1940.
4. Kirk, Esben: Gasometric Microdetermination of Phosphoric Acid, J. Biol. Chem. **106**: 191, 1934.
5. Hoagland, Charles L.: Microdetermination of Sulfate and Phosphate by Manometric Combustion of Their Organic Precipitates, J. Biol. Chem. **136**: 543, 1940.
6. Van Slyke, Donald D., and Kreysa, Frank J.: Microdetermination of Calcium by Precipitation as Pierolonate and Estimation of the Precipitated Carbon by Manometric Combustion, J. Biol. Chem. **142**: 765, 1942.
7. Hoagland, Charles L.: Micromanometric Determination of Magnesium, J. Biol. Chem. **136**: 553, 1940.
8. Macy, Icie G.: Nutrition and Chemical Growth in Childhood, Vol. 1, Evaluation, Springfield, Ill., 1942, Charles C Thomas.
9. Schoenheimer, Rudolf, and Sperry, Warren M.: A Micromethod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**: 745, 1934.
10. Sperry, Warren M.: A Micromethod for the Determination of Total and Free Cholesterol, Am. J. Clin. Path. Vol. 8, Technical Supplement, **2**: 91, 1938.

A MECHANICAL MANIPULATOR FOR SMALL PIPETTES*

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NEW YORK, N. Y.

IN THE course of some work on the chemical properties of native mucous secretion from the digestive tract, we encountered difficulty in delivering exact volumes of this extremely viscous material for microchemical analysis. Our analytical methods aim at a precision of better than 1 per cent, and they call for the delivery of 0.1 ml. samples with a considerable degree of reproducibility.

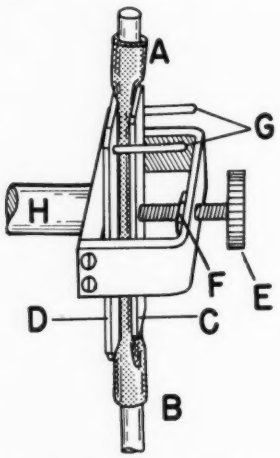

Fig. 1.—Manipulator for small pipettes.

Because of its extreme viscosity and tenacity, we found it necessary to draw the material up into the sampling pipette without overshooting the graduation as well as to deliver the sample with rinsing. Consequently, the usual oral method of manipulating a pipette was unsuited for this purpose, and it became necessary to employ a mechanical device. The following arrangement, embodying a simple mechanical principle, has proved so very efficient in our work, as well as for other purposes, that we describe it for the benefit of others, especially workers in clinical laboratories who frequently work with 0.1 ml. quantities.

Contrary to the usual procedure, the pipette and manipulator are supported rigidly in a ring stand. The vessel containing the material for analysis is supported underneath by one hand while the sample is being taken. Suction and delivery are both effected by mechanically controlled changes in the volume of air trapped in a small piece of rubber tubing. The upper end of this tubing (*A*) is closed with a piece of glass rod or with rubber cement; the pipette stem is at-

*From the Laboratories of the Mount Sinai Hospital, New York City.
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tached to its lower end at (*B*). The tubing is held between two rigid metal plates, the front one of which (*C*) can be driven gradually against the back one (*D*) by means of a screw with knurled knob (*E*). These plates are both 5 cm. long; their widths are 13 and 35 mm., respectively. The distance between the front of the supporting frame and the back plate is about 15 mm. A nut (*F*) soldered to the inside of the supporting frame serves to steady the movement of the screw without increasing the weight of the device. The pins (*G*) serve only to keep the front plate (*C*) from swerving from side to side, and the 15 cm. rod (*H*) supports the device on a ring stand.

Before the pipette is immersed in the liquid, the tubing must be compressed to a moderate degree; thereafter, gradual release of this pressure will serve to draw the liquid up to the graduation mark without overshooting the mark, and recompression will serve to deliver the sample as required. In order to insure complete filling and emptying of the pipette, the volume of air contained in the rubber tubing must be slightly greater than the capacity of the pipette. For 0.1 ml. samples we use a piece of red rubber tubing (inside diameter 4.5 mm., wall thickness 1 mm.) about 6 cm. long, exclusion of the upper and lower ends which are filled with glass. A similar device of slightly larger dimensions has been employed for pipettes of greater capacity.

SIMPLIFIED MICROMETHOD FOR BLOOD SULFONAMIDE DETERMINATION*

GILBERT M. JORGENSEN, M.D., ANCON, CANAL ZONE

IN THIS report is described a modification of the diazotization method[1] for quantitative determination of unconjugated sulfonamides in blood. The modification permits the use of smaller quantities of blood and lessens the time required for performance of the test. In accomplishing these advantages only a small degree of accuracy has been sacrificed.

A reduction in the amount of blood required to only 0.02 c.c. has made possible blood sulfonamide determinations on small laboratory animals without sacrificing their lives. The method has been applied without difficulty for blood sulfonamide determinations on mice. It has been used for six months for routine determinations on children receiving sulfonamides, and has proved satisfactory.

A saving in time required for performance of the test has been accomplished by omitting the precipitation of blood proteins which permits the determination to be carried out in one vessel.

An Evelyn photoelectric colorimeter with filter 540 M has been employed, though other photoelectric colorimeters should serve equally well. For measuring the blood ordinary 0.02 c.c. "hemoglobin" pipettes have been used.

*From the Department of Pediatrics, Columbia Medical School, and the Babies Hospital, New York.

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The reagents required are glacial acetic acid, 0.1 per cent aqueous solution of sodium nitrite, 0.5 per cent aqueous solution of ammonium sulfamate, and a 1 per cent aqueous solution of N-(naphthyl) ethylenediamine dihydrochloride.

PROCEDURE

To 2 c.c. of distilled water in a colorimeter tube add 0.02 c.c. of capillary blood with a "hemoglobin" pipette. Add 2 c.c. of glacial acetic acid and 1 c.c. of a 0.1 per cent aqueous solution of sodium nitrite. Shake. After one minute or more add 1 c.c. of a 0.5 per cent aqueous solution of ammonium sulfamate. Shake. As soon as the bubbles have risen to the surface, insert the tube into the Evelyn photoelectric colorimeter after adjusting the tube holder for 6 c.c. volumes and inserting filter 540 M. To obtain the first of the two readings necessary, adjust the galvanometer string to 5.0 on the lower scale (the midpoint, sometimes designated 50), remove the tube, and read the galvanometer. This first reading (air setting) serves as a blank and also enables one to calculate the hemoglobin concentration.

After the first reading has been obtained, add 0.02 c.c. of a 1 per cent aqueous solution of N-(naphthyl) ethylenediamine dihydrochloride from a dropper calibrated to deliver that amount per drop. Shake. After three minutes or more adjust the galvanometer string to the first reading, insert the tube, and obtain the second reading.

Calculation of the concentration of the sulfonamide present involves the use of the following formula:

Concentration of sulfonamide = K (log 5.0 – log second reading) + C. K is a constant determined by using a hemoglobin-free solution of a sulfonamide of known concentration. C is a correction made necessary because of the presence of hemoglobin; within the range of hemoglobin values encountered, the correction necessary is approximately proportional to the hemoglobin concentration.

The concentration of hemoglobin may be calculated from the following formula:

Concentration of hemoglobin = K′ (log first reading – log first reading of hemoglobin-free blank).

The correction C = K (log 5.0 – log second reading) K″ (log first reading – log first reading of hemoglobin-free blank) may be incorporated into the first formula as follows:

Concentration of sulfonamide = K (log 5.0 – log second reading) + K (log 5.0 – log second reading) K″ (log first reading – log first reading of hemoglobin-free blank). K, K′, and K″ may be determined from standards of known concentration. The application of the above formula appears rather laborious; however, with the aid of a calculating machine one may prepare in a few hours a table listing the values for all galvanometer readings.

The correction, C, is not large; for sulfanilamide it amounts to about 1 per cent per gram of hemoglobin. For routine determinations, if an error up to 10 per cent is acceptable, one may simplify the standardization and the prepara-

tion of a reference table by omitting C from the formula and determining a constant K‴, using blood of average hemoglobin concentration in addition to a standard sulfonamide solution. A blank is not necessary except for the determination of the constants.

The most likely source of error results from the occasional adherence of bubbles to the side of the colorimeter tube. If the tubes are cleaned with cleaning solution, bubbles seldom adhere to the glass, and when present, they usually may be dislodged by shaking the tube.

REFERENCE

1. Bratton, A. C., and Marshall, E. K., Jr.: New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537-550, 1939.

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ITEM

Meeting of Scientific Photographers

The Biological Photographic Association will hold its Twelfth Annual Convention in New York City September 10, 11, and 12. Present-day methods of obtaining photographs for teaching and scientific records will be discussed in detail. Particular emphasis will be placed on the types of photographs needed in the present emergency. The Convention Chairman will be Mr. Joseph Haulenbeek, Illustration Division, Rockefeller Institute for Medical Research, New York City. Further particulars about the meeting and the program may be had by writing him.

The Biological Photographic Association is a national, nonprofit organization, formed to act as a clearing house for information on the methods of scientific illustration. Its members are scientific photographers, scientists interested in the application of photography to their fields, and the makers of precision photographic equipment. Information on various techniques, collected from experienced workers all over the country, is dispensed by means of the Association's Quarterly Journal, Travelling Loan Albums and Print Salons, and direct correspondence through the office of the Secretary. The 1942 Salon of medical and biological prints will be on view at the Convention.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

HEMOGLOBINOMETRY, Rimington, C. Brit. M. J. 1: 177, 1942.

The following method is described:

A 1:500 dilution of blood in decinormal sodium hydroxide is made, using a standard or calibrated pipette. This dilution may, of course, be made in two steps, if it is preferred, as in the worked example quoted below. After a few minutes at room temperature a convenient volume, such as 10 c.c., is measured into a small flask, and one-fifth of the volume of pyridine is added. Upon mixing by gentle rotation the conversion of the hemepigments into the alkaline parahematin is completed within a few seconds; hence there is no necessity to allow the original blood dilution to stand for ten, twenty, or even forty minutes before proceeding, or alternatively to heat in a boiling water bath, as is required when the alkaline hematin method is used.

A pinch of good quality sodium hydrosulfite ($Na_2S_2O_4$) is now added to the mixture, and the contents of the flask are swirled gently until the solid has dissolved. The immediate color change to pink denotes the conversion of the pigments into pyridine hemochromogen. Without undue delay (to guard against reoxidation) the solution is transferred to a photoelectric colorimeter cell, and the light absorption in the region 550 to 555 mμ is measured, using a suitable filter (e.g., Ilford spectrum filter No. 605—yellow green). From the calibration factor, obtained by using known weights of pure hemin instead of blood and proceeding as above, the hemoglobin content of the blood is then calculated.

Molecular weight of hemin, $C_{34}H_{32}O_4N_4FeCl = 651.4$

Iron content of hemoglobin = 0.334 per cent

Therefore, Molecular weight of hemoglobin = 66,890

$$\frac{\text{Hemoglobin}}{\text{Hemin}} = \frac{66{,}890}{4 \times 651.4} = 25.66$$

Should it be contended that the error involved in calculating the molecular weight of hemoglobin from its iron content is undesirably large, the results might alternatively be expressed in terms of hemin or hematin. The extension of pyridine-hemochromogen prepared from crystalline hemin and using a photoelectric absorptiometer with Ilford No. 605 filter and 1 cm. cell was found to be 0.108 at a concentration of 1 mg. per 100 c.c.

Should no photoelectric colorimeter be available, the concentration of the pyridine-hemochromogen solution may be determined by one of the alternative methods, such as color comparison in a colorimeter with a standard prepared from hemin (standard solutions of hemin in alkali should be kept in the dark) or dilution while viewing with a hand spectroscope until the intensity of the absorption band matches that of a standard, as described by Roets (1940). This last method is certainly the most simple and rapid, although not quite so accurate as the use of a colorimeter. Pure hemin is easily prepared according to Gattermann (1937), but should be recrystallized as follows:

Five grams of hemin are dissolved in 15 c.c. of pyridine, and 40 c.c. of chloroform are added. The solution is passed through a No. 3 or No. 4 sintered-glass filter to remove any protein and is run into a mixture of 250 c.c. of acetic acid plus 5 c.c. of saturated sodium chloride plus 4 c.c. of concentrated hydrochloric acid, which is maintained at water-bath temperature. After cooling to room temperature the crystals are filtered and washed successively with 50 per cent acetic acid, water, alcohol, and ether, and then dried.

The author has used the pyridine-hemochromogen method with equal success for specimens of plasma and urine containing hemoglobin, myohemoglobin, or mixtures of these with their derivatives. It is only necessary to adjust the initial dilution so that the concentration of pigment in the final solution falls within the comfortable range for measurement.

Example: 1 c.c. of blood was diluted to 100 c.c. with decinormal sodium hydroxide. To 1 c.c. of this solution was added 3 c.c. of the alkalin, then 1 c.c. of pyridine and, after mixing, a pinch of sodium hydrosulfite. In a 1 cm. cell the reading on the Hilger-Nutting photometer at 552 mμ was 0.573. The calibration for this instrument had been found to be 0.62. Hence:

$$\text{Hb. per c.c. of blood} = 0.573 \times 0.62 \times 500 =$$
$$177.6 \text{ mg. per c.c., i.e., } 17.76 \text{ per cent}$$

The same result was obtained whether the determination was completed within five minutes or after allowing the mixture with alkali to stand for one hour and that with pyridine for a further half hour.

B. DYSENTERIAE (Flexner), Experience With the Wilson and Blair Medium for, Bradshaw, D. B. Brit. M. J. **1**: 181, 1942.

Table of results with Wilson and Blair medium and those with MacConkey's Agar:

Case No.	Acute or Convalescent	No. of Flexner Colonies		Remarks
		MacConkey Plate	Wilson-Blair Plate	
240	A	1	1	
246	A	+	+	
259	C	Negative	+	
262	C	Negative	+	
267	A	20	Uncountable	Pure culture of Flexner on Wilson-Blair plate
271	C	10	Uncountable	
272	C	2	10	
275	A	Negative	Uncountable	Pure cultures of Flexner on Wilson-Blair plate
284	A	Negative	Uncountable	
285	A	Uncountable	Uncountable	Little to choose between the two media
298	C	Negative	Uncountable	
300	C	Negative	Uncountable	
341	C	9	Negative	
342	C	1	Negative	
343	C	9	100	
353	C	1	30	
372	C	Negative	Uncountable	Pure culture of Flexner on Wilson-Blair medium
383	C	Negative	30	Pure culture of Flexner on Wilson-Blair medium
384	C	Negative	100	Pure culture of Flexner on Wilson-Blair medium
392	C	Negative	10	

Positive on both media: 8
Positive on Wilson and Blair's medium only: 10
Positive on MacConkey agar only: 2
Total positive on Wilson and Blair's medium: 19 (90 per cent)
Total positive on MacConkey's agar: 10 (50 per cent)

TAKATA-ARA REACTION, the Diagnostic Value of, Waugh, T. R., and McKenna, R. D. Am. J. M. Sc. **203**: 722, 1942.

Analysis of a series of 300 Takata-Ara reactions, carried out for the most part on patients who showed a hyperbilirubinemia or who gave reason to suspect the presence of hepatic disease, indicates that the test has considerable diagnostic value. It would appear that the difference of opinion which has arisen in the literature concerning its value is due to the use of various techniques and a lack of precision in reading the results. A means is described whereby more accurate results may be obtained.

The method used by the authors follows:

The test may be done on blood serum, plasma, or transudates such as ascitic fluid. Most of our results were obtained from oxalated plasma procured at the time of carrying out

a complete hemogram. Blood is usually taken for such tests in the morning, between 10 and 12 o'clock, to avoid any effect of digestive processes on the plasma. At least 1 c.c., free from hemolysis and cells, is required.

A series of 8 tubes, approximately 10 cm. in length and the inner bore of 1 cm., are placed in a rack. The tubes must, of course, be thoroughly clean. One cubic centimeter of 0.9 per cent sodium chloride is pipetted into each tube, and 1 c.c. of plasma is added to the first tube. This is mixed with the saline by shaking, and 1 c.c. of the mixture is withdrawn and placed in the second tube. By continuing in this manner and discarding 1 c.c. from the last tube a series of increasing dilutions (1:2 to 1:256) of plasma in saline is set up. To each tube, first 0.25 c.c. of a 10 per cent solution of sodium carbonate and then 0.15 c.c. of 0.5 per cent bichloride of mercury is added. Complete mixing is obtained by shaking after each solution has been placed in the tube. A slight precipitate, which disappears on shaking, may occur after addition of the mercury. The tubes are then allowed to stand at room temperature for twenty-four hours.

At the end of this interval the result of the test is read by estimating the amount of precipitate in each tube. This is expressed as 0, T (trace), 1, 2, 3, or 4. Precipitates classed as "4" occupy approximately the lower third of the solution in the tube. In doubtful cases, accurate reading of the amount of precipitate may be accomplished by thoroughly shaking up the tube and filling a Wintrobe sedimentation tube to the 10 cm. mark with a portion of the contents. The Wintrobe tube is then centrifugalized at high speed for fifteen minutes. Less than 1 mm. of whitish sediment in the bottom of the tube is classed as a trace (T), 1 to 2 mm. as "1," 2 to 3 mm. as "2," and so on. Any amount over 4 mm. is designated "4."

The Takata-Ara reaction is positive when two adjacent tubes show a grade "4" degree of precipitate and the next tube is at least a grade "3," for example (00T2441) or (00134421). Plus-minus reactions are reported whenever the criteria for the positive reaction are not reached, but at least two tubes show a grade "3" sediment, for example (00T12431), (0001331), or (000TT332). All reactions of lower degree are negative as (000TT231) or (000TT1TT). It would appear that accurate reading of the reaction is extremely important.

C. DIPHTHERIAE, Further Studies on the Serological Classification of, Huang, C. H. Am. J. Hyg. 35: 335, 1942.

Eight serologic types and an unidentified remainder have been found among the diphtheria bacilli isolated from the throats of 268 clinical patients with diphtheria.

Avirulent organisms, besides the virulent ones, were occasionally found in diphtheria patients in the acute and convalescent stages. In no instance was an avirulent organism found which was at the same time agglutinable by our 8-type antisera, affording, therefore, no evidence for the transformation of a virulent diphtheria organism into an avirulent one in the throat of diphtheria patients.

The failure to find an avirulent organism, which is at the same time agglutinable by 8-type antisera in acute and convalescent cases, together with the consistent finding of virulence in all the diphtheria organisms which belonged serologically to the author's 8 types, suggested the possibility of using the serologic agglutination as an indirect, but more convenient, means of determining the virulence of organisms in acute and convalescent cases.

Laryngeal diphtheria is less frequently observed in patients who were infected by type D_1 organisms than with other types of organisms. It happened that type D_1 is the only type of diphtheria organisms which possesses both gravis and intermedius strains.

The prevalence of types of organisms varies in different years.

Simultaneous and repeated swabs from the throat of the same patient practically always revealed the same type of organism. Only two patients were found to be infected by two different types of virulent organisms at the same time.

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